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(54) Title: IMMUNOGENIC COMPOSITIONS FOR *STREPTOCOCCUS AGALACTIAE*

(57) Abstract: This application relates to Group B Streptococcus ("GBS") vaccines comprising combinations of GBS polypeptide antigens where the polypeptides contribute to the immunological response in a recipient. Preferably, the compositions of the invention comprise a combination of two or more GBS antigens, wherein said combination includes GBS 80 or a fragment thereof. In one embodiment, the combination may consist of two to thirteen GBS antigens selected from an antigen group consisting of GBS 80, GBS 91, GBS 104, GBS 184, GBS 276, GBS 305, GBS 322, GBS 330, GBS 338, GBS 361, GBS 404, GBS 690, and GBS 691.



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**IMMUNOGENIC COMPOSITIONS FOR *STREPTOCOCCUS AGALACTIAE***

This application claims the benefit and incorporates by reference in its entirety U.S. provisional application 60/548,789, filed February 26, 2004 and claims priority to International Patent Application No. PCT/US03/29167, Attorney Reference No. PP19766.002, filed on September 15, 2003, incorporated herein in its entirety.

**FIELD OF THE INVENTION**

The invention relates to an immunogenic antigen derived from *Streptococcus agalactiae* (“GBS”) and its use in combinations with other GBS antigens to provide for broader coverage among different GBS strains. In particular, the invention relates to a composition comprising a combination of two or more GBS antigens, wherein the combination includes GBS 80 or a fragment thereof. The combination may include GBS 80 and at least one other GBS antigen. For example, the combination may include GBS 80 and up to thirteen GBS antigens. In a preferred embodiment, the combination may include GBS 80 and up to ten GBS antigens. In a more preferred embodiment, the combination may include GBS 80 and up to five GBS antigens. In one embodiment, the combination may consist of two to thirteen GBS antigens selected from an antigen group consisting of GBS 80, GBS 91, GBS 104, GBS 184, GBS 276, GBS 305, GBS 322, GBS 330, GBS 338, GBS 361, GBS 404, GBS 690, and GBS 691. Preferably, the combination includes GBS 80 in combination with one or more of GBS 104 and GBS 322.

**BACKGROUND OF THE INVENTION**

GBS has emerged in the last 20 years as the major cause of neonatal sepsis and meningitis that affect 0.5 – 3 per 1000 live births, and an important cause of morbidity among the older age group affecting 5 – 8 per 100,000 of the population. Current disease management strategies rely on intrapartum antibiotics and neonatal monitoring which have reduced neonatal case mortality from >50% in the 1970’s to less than 10% in the 1990’s. Nevertheless, there is still considerable morbidity and mortality and the management is expensive. 15 – 35% of pregnant women are asymptomatic carriers and at high risk of transmitting the disease to their babies. Risk of neonatal infection is associated with low serotype specific maternal antibodies and high titers are believed to be protective. In addition, invasive GBS disease is increasingly recognized in elderly adults with underlying disease such as diabetes and cancer.

The “B” in “GBS” refers to the Lancefield classification, which is based on the antigenicity of a carbohydrate which is soluble in dilute acid and called the C carbohydrate. Lancefield identified 13 types of C carbohydrate, designated A to O, that could be serologically differentiated. The organisms

that most commonly infect humans are found in groups A, B, D, and G. Within group B, strains can be divided into at least 9 serotypes (Ia, Ib, Ia/c, II, III, IV, V, VI, VII and VIII) based on the structure of their polysaccharide capsule. In the past, serotypes Ia, Ib, II, and III were equally prevalent in normal vaginal carriage and early onset sepsis in newborns. Type V GBS has emerged as an important cause of GBS infection in the USA, however, and strains of types VI and VIII have become prevalent among Japanese women.

The genome sequence of a serotype V strain 2603 V/R has been published (Ref. 1) and various polypeptides for use as vaccine antigens have been identified (Ref. 2). The vaccines currently in clinical trials, however, are based on polysaccharide antigens. These suffer from serotype-specificity and poor immunogenicity, and so there is a need for effective vaccines against *S. agalactiae* infection.

It is an object of the invention to provide further and improved compositions for providing immunity against GBS disease and/or infection. The compositions are based on a combination of two or more (e.g., three or more) GBS antigens.

## SUMMARY OF THE INVENTION

Applicants have discovered that an immunogenic GBS antigen, GBS 80, is particularly suitable for immunization purposes, especially when used in combination with other GBS antigens. The combination may include GBS 80 and at least one other GBS antigen or up to thirteen other GBS antigens. In a preferred embodiment, the combination may include GBS 80 and up to 10 GBS antigens. In a more preferred embodiment, the combination includes GBS 80 and up to five GBS antigens. In particular, the invention relates to a composition comprising a combination of two or more GBS antigens, wherein the combination includes GBS 80 or a fragment thereof. In one embodiment, the combination may consist of two to thirteen GBS antigens selected from the group consisting of GBS 80, GBS 91, GBS 104, GBS 184, GBS 276, GBS 305, GBS 322, GBS 330, GBS 338, GBS 361, GBS 404, GBS 690, and GBS 691. Preferably, the combination consists of GBS 80, GBS 104 and GBS 322.

Instead of the full length antigen, the combination may comprise an immunogenic fragment of the selected GBS antigen and/or a polypeptide sequence having sequence identity to the selected antigen.

Preferably, the combination of GBS antigens consists of three, four, five, six, seven, eight, nine, or ten GBS antigens. Still more preferably, the combination of GBS antigens consists of three, four, or five GBS antigens.

### DETAILED DESCRIPTION OF THE INVENTION

The practice of the present invention will employ, unless otherwise indicated, conventional methods of chemistry, biochemistry, molecular biology, immunology and pharmacology, within the skill of the art. Such techniques are explained fully in the literature. See, e.g., *Remington's Pharmaceutical Sciences*, Mack Publishing Company, Easton, Pa., 19th Edition (1995); *Methods In Enzymology* (S. Colowick and N. Kaplan, eds., Academic Press, Inc.); and *Handbook of Experimental Immunology*, Vols. I-IV (D.M. Weir and C.C. Blackwell, eds., 1986, Blackwell Scientific Publications); Sambrook, et al., *Molecular Cloning: A Laboratory Manual* (2nd Edition, 1989); *Handbook of Surface and Colloidal Chemistry* (Birdi, K.S. ed., CRC Press, 1997); *Short Protocols in Molecular Biology*, 4th ed. (Ausubel et al. eds., 1999, John Wiley & Sons); *Molecular Biology Techniques: An Intensive Laboratory Course*, (Ream et al., eds., 1998, Academic Press); *PCR (Introduction to Biotechniques Series)*, 2nd ed. (Newton & Graham eds., 1997, Springer Verlag); Peters and Dalrymple, *Fields Virology* (2d ed), Fields et al. (eds.), B.N. Raven Press, New York, NY.

All publications, patents and patent applications cited herein, are hereby incorporated by reference in their entireties.

#### GBS Antigens

5 As discussed above, the invention provides an immunogenic composition comprising a combination of two or more GBS antigens, wherein said combination includes GBS 80 or a fragment thereof.

The combinations of GBS antigens may include polypeptide fragments of the identified GBS antigens. The length of the fragment may vary depending on the amino acid sequence of the specific  
 10 GBS antigen, but the fragment is preferably at least 7 consecutive amino acids, (e.g. 8, 10, 12, 14, 16, 18, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90, 100, 150, 200 or more). Preferably the fragment comprises one or more epitopes from the sequence. Other preferred fragments include (1) the N-terminal signal peptides of each identified GBS antigen, (2) the identified GBS antigens without their N-terminal signal peptides, and (3) each identified GBS antigen wherein up to 10 amino acid residues (e.g. 1, 2,  
 15 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) are deleted from the N-terminus and/or the C-terminus e.g. the N-terminal amino acid residue may be deleted. Other fragments omit one or more domains of the protein (e.g. omission of a signal peptide, of a cytoplasmic domain, of a transmembrane domain, or of an extracellular domain).

The combinations of GBS antigens may include polypeptide sequences having sequence  
 20 identity to the identified GBS antigens. The degree of sequence identity may vary depending on the amino acid sequence (a) in question, but is preferably greater than 50% (e.g. 60%, 65%, 70%, 75%,

80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5% or more). Polypeptides having sequence identity include homologs, orthologs, allelic variants and functional mutants of the identified GBS antigens. Typically, 50% identity or more between two proteins is considered to be an indication of functional equivalence. Identity between proteins is preferably determined by the Smith-Waterman homology search algorithm as implemented in the MPSRCH program (Oxford Molecular), using an affinity gap search with parameters *gap open penalty=12* and *gap extension penalty=1*.

The polypeptides can, of course, be prepared by various means (*e.g.* recombinant expression, purification from GBS, chemical synthesis *etc.*) and in various forms (*e.g.* native, fusions, glycosylated, non-glycosylated *etc.*). They are preferably prepared in substantially pure form (*i.e.* substantially free from other streptococcal or host cell proteins) or substantially isolated form.

#### GBS 80

As discussed above, the invention relates to the use of GBS 80 in synergistic combination with other GBS antigens. GBS 80 refers to a putative cell wall surface anchor family protein.

Nucleotide and amino acid sequence of GBS 80 sequenced from serotype V isolated strain 2603 V/R are set forth in Ref. 2 as SEQ ID 8779 and SEQ ID 8780. These sequences are also set forth below as SEQ ID NOS 1 and 2:

#### **SEQ ID NO. 1**

ATGAAATTATCGAAGAAGTTATTGTTTTTCGGCTGCTGTTTTTAACAATGGTGGCGGGGTCAACTGTTGA  
ACCAGTAGCTCAGTTTGC GACTGGAATGAGTATTGTAAGAGCTGCAGAAGTGTCACAAGAACGCCAG  
CGAAAACAACAGTAAATATCTATAAATTACAAGCTGATAGTTATAAATCGGAAATTACTTCTAATGGT  
GGTATCGAGAATAAAGACGGCGAAGTAATATCTAACTATGCTAACTTGGTGACAATGTAAAAGGTTT  
GCAAGGTGTACAGTTTAAACGTTATAAAGTCAAGACGGATATTCTGTTGATGAATTGAAAAATTGA  
CAACAGTTGAAGCAGCAGATGCAAAAGTTGGAACGATTCTTGAAGAAGGTGTCAGTCTACCTCAAAAA  
ACTAATGCTCAAGGTTTGGTTCGTCGATGCTCTGGATTCAAAAAGTAATGTGAGATACTTGTATGTAGA  
AGATTTAAAGAATTCACCTTCAAACATTACCAAAGCTTATGCTGTACCGTTTGTGTTGGAATTACCAG  
TTGCTAACTCTACAGGTACAGGTTTCTTTCTGAAATTAATATTTACCCTAAAAACGTTGTAAGTATGAT  
GAACCAAAAACAGATAAAGATGTTAAAAAATTAGGTGAGGACGATGCAGGTTATACGATTGGTGAAGA  
ATTCAAATGGTTCTTGAAATCTACAATCCCTGCCAATTTAGGTGACTATGAAAAATTTGAAATTACTG  
ATAAATTTGCAGATGGCTTGACTTATAAATCTGTTGGAAAAATCAAGATTGGTTCGAAAACACTGAAT  
AGAGATGAGCACTACACTATTGATGAACCAACAGTTGATAACCAAAATACATTAAAAATTACGTTTAA  
ACCAGAGAAATTTAAAGAAATTGCTGAGCTACTTAAAGGAATGACCCTTGTTAAAAATCAAGATGCTC  
TTGATAAAGCTACTGCAAATACAGATGATGCGGCATTTTTGGAATTCAGTTGCATCAACTATTAAT  
GAAAAAGCAGTTTTTAGGAAAAGCAATTGAAAATACTTTTGAAGTTCAATATGACCATACTCCTGATAA  
AGCTGACAATCCAAAACCATCTAATCCTCCAAGAAAACCAGAAGTTCATACTGGTGGGAAACGATTTG  
TAAAGAAAGACTCAACAGAAACACAAACACTAGGTGGTGTGAGTTTGATTTGTTGGCTTCTGATGGG  
ACAGCAGTAAATGGACAGATGCTCTTATTAAAGCGAATACTAATAAAAACATATTTGCTGGAGAAGC  
TGTTACTGGGCAACCAATCAAATTGAAATCACATACAGACGGTACGTTTGAGATTAAAGGTTTGGCTT  
ATGCAGTTGATGCGAATGCAGAGGGTACAGCAGTAACCTACAAATTTAAAGAAACAAAAGCACCAGAA  
GGTTATGTAATCCCTGATAAAGAAATCGAGTTTACAGTATCACAAACATCTTATAATACAAAACCAAC  
TGACATCACGGTTGATAGTGCTGATGCAACACCTGATACAATTTAAAAACAACAAACGTCCTTCAATCC  
CTAATACTGGTGGTATTGGTACGGCTATCTTTGTCGCTATCGGTGCTGCGGTGATGGCTTTTGCTGTT  
AAGGGGATGAAGCGTCGTACAAAAGATAAC

**SEQ ID NO: 2**

MKLSKKLLFSAAVLTMVAGSTVEPVAQFATGMSIVRAAEVSQERPAKTTVNIYKLQADSYKSEITSNG  
 GIENKDGEVISNYAKLGDNVKGLQGVQFKRYKVKTDISVDELKKLTVEAADAKVGTILEEGVSLPQK  
 5 TNAQGLVVDALDSKSNVRYLYVEDLKNSPSNITKAYAVPFVLELPVANSTGTGFLSEINIYPKNVVT  
 EPKTDKDVKKLGQDDAGYTIGEEFKWFLKSTIPANLGDYEKFEITDKFADGLTYKSVGKIKIGSKTLN  
 RDEHYTIDEPTVDNQNTLKITFKPEKFKEIAELLKGMTLVKNQDALDKATANTDDAAFLEIPVASTIN  
 EKAVLGKAIENTFELQYDHTPDKADNPKPSNPPRKPEVHTGGKRFVKKDSTETQTLGGAEFDLLASDG  
 10 TAVKWTDALIKANTNKNYIAGEAVTGQPIKLKSHTDGTFEIKGLAYAVDANAEGTAVTYKLKETKAPE  
 GYVIPDKEIEFTVSQTSYNTKPTDITVDSADATPDTIKNNKRPS IPNTGGIGTAIFVAIGAAMFAV  
 KGMKRRTKDN

As described above, the combinations of the invention may include a fragment of a GBS  
 antigen. In some instances, removal of one or more domains, such as a leader or signal sequence  
 15 region, a transmembrane region, a cytoplasmic region or a cell wall anchoring motif, may facilitate  
 cloning of the gene encoding the antigen and/or recombinant expression of the GBS protein. In  
 addition, fragments comprising immunogenic epitopes of the cited GBS antigens may be used in the  
 compositions of the invention.

GBS 80 contains an N-terminal leader or signal sequence region which is indicated by the  
 20 underlined sequence at the beginning of SEQ ID NO: 2 above. In one embodiment, one or more  
 amino acids from the leader or signal sequence region of GBS 80 are removed. An example of such a  
 GBS 80 fragment is set forth below as SEQ ID NO: 3:

**SEQ ID NO: 3**

AEVSQERPAKTTVNIYKLQADSYKSEITSNGGIENKDGEVISNYAKLGDNVKGLQGVQFKRYKVKT  
 25 SVDELKKLTVEAADAKVGTILEEGVSLPQKTNAQGLVVDALDSKSNVRYLYVEDLKNSPSNITKAYA  
 VPFVLELPVANSTGTGFLSEINIYPKNVVTDEPKTDKDVKKLGQDDAGYTIGEEFKWFLKSTIPANLG  
 DYEKFEITDKFADGLTYKSVGKIKIGSKTLNRDEHYTIDEPTVDNQNTLKITFKPEKFKEIAELLKGM  
 30 TLVKNQDALDKATANTDDAAFLEIPVASTINEKAVLGKAIENTFELQYDHTPDKADNPKPSNPPRKPE  
 VHTGGKRFVKKDSTETQTLGGAEFDLLASDGTAVKWTDALIKANTNKNYIAGEAVTGQPIKLKSHTDG  
 TFEIKGLAYAVDANAEGTAVTYKLKETKAPEGYVIPDKEIEFTVSQTSYNTKPTDITVDSADATPDTI  
 KNNKRPSIPNTGGIGTAIFVAIGAAMFAV KGMKRRTKDN

GBS 80 contains a C-terminal transmembrane region which is indicated by the underlined  
 35 sequence near the end of SEQ ID NO: 2 above. In one embodiment, one or more amino acids from  
 the transmembrane region and/or a cytoplasmic region are removed. An example of such a GBS 80  
 fragment is set forth below as SEQ ID NO: 4:

**SEQ ID NO: 4**

MKLSKKLLFSAAVLTMVAGSTVEPVAQFATGMSIVRAAEVSQERPAKTTVNIYKLQADSYKSEITSNG  
 40 GIENKDGEVISNYAKLGDNVKGLQGVQFKRYKVKTDISVDELKKLTVEAADAKVGTILEEGVSLPQK  
 TNAQGLVVDALDSKSNVRYLYVEDLKNSPSNITKAYAVPFVLELPVANSTGTGFLSEINIYPKNVVT  
 EPKTDKDVKKLGQDDAGYTIGEEFKWFLKSTIPANLGDYEKFEITDKFADGLTYKSVGKIKIGSKTLN  
 45 RDEHYTIDEPTVDNQNTLKITFKPEKFKEIAELLKGMTLVKNQDALDKATANTDDAAFLEIPVASTIN  
 EKAVLGKAIENTFELQYDHTPDKADNPKPSNPPRKPEVHTGGKRFVKKDSTETQTLGGAEFDLLASDG

TAVKWTDALIKANTNKNYIAGEAVTGQPIKLKSHTDGTFEIKGLAYAVDANAEGTAVTYKLKETKAPE  
GYVIPDKEIEFTVSQTSYNTKPTDITVDSADATPDTIKNNKRPS *IPNTG*

GBS 80 contains an amino acid motif indicative of a cell wall anchor: **SEQ ID NO: 5**

**5** *IPNTG* (shown in italics in SEQ ID NO: 2 above). In some recombinant host cell systems, it may be preferable to remove this motif to facilitate secretion of a recombinant GBS 80 protein from the host cell. Accordingly, in one preferred fragment of GBS 80 for use in the invention, the transmembrane and/or cytoplasmic regions and the cell wall anchor motif are removed from GBS 80. An example of such a GBS 80 fragment is set forth below as SEQ ID NO: 6.

**SEQ ID NO: 6**

MKLSKKLLFSAAVLTMVAGSTVEPVAQFATGMSIVRAAEVSQERPAKTTVNIYKLQADSYKSEITSNG  
GIENKDGEVISNYAKLGDNVKGLQGVQFKRYKVKTDISVDELKKLTTVEAADAKVGTILEEGVSLPQK  
TNAQGLVVDALDSKSNVRYLYVEDLKNSPSNITKAYAVPFVLELPPVANSTGTGFLSEINIYPKNVVT  
**15** EPKTDKDVKKLGQDDAGYTI GEEFKWFLKSTIPANLG DYEFKFEITDKFADGLTYKSVGKIKIGSKTLN  
RDEHYTIDEPTVDNQNTLKITFKPEKFKEIAELLKGMTLVKNQDALDKATANTDDAAFLEIPVASTIN  
EKAVLGKAIENFELQYDHTPDKADNPKNPNPPRKPEVHTGGKRFVKKDSTETQTLGGAFFDLLASDG  
TAVKWTDALIKANTNKNYIAGEAVTGQPIKLKSHTDGTFEIKGLAYAVDANAEGTAVTYKLKETKAPE  
GYVIPDKEIEFTVSQTSYNTKPTDITVDSADATPDTIKNNKRPS

**20** Alternatively, in some recombinant host cell systems, it may be preferable to use the cell wall anchor motif to anchor the recombinantly expressed protein to the cell wall. The extracellular domain of the expressed protein may be cleaved during purification or the recombinant protein may be left attached to either inactivated host cells or cell membranes in the final composition.

**25** In one embodiment, the leader or signal sequence region, the transmembrane and cytoplasmic regions and the cell wall anchor motif are removed from the GBS 80 sequence. An example of such a GBS 80 fragment is set forth below as SEQ ID NO: 7.

**SEQ ID NO: 7**

**30** AEVSQERPAKTTVNIYKLQADSYKSEITSNGGIENKDGEVISNYAKLGDNVKGLQGVQFKRYKVKT  
SVDELKKLTTVEAADAKVGTILEEGVSLPQKTNAQGLVVDALDSKSNVRYLYVEDLKNSPSNITKAYA  
VPFVLELPPVANSTGTGFLSEINIYPKNVVTDEPKTDKDVKKLGQDDAGYTI GEEFKWFLKSTIPANLG  
DYEFKFEITDKFADGLTYKSVGKIKIGSKTLNRDEHYTIDEPTVDNQNTLKITFKPEKFKEIAELLKGM  
TLVKNQDALDKATANTDDAAFLEIPVASTINEKAVLGKAIENFELQYDHTPDKADNPKNPNPPRKPE  
**35** VHTGGKRFVKKDSTETQTLGGAFFDLLASDGTAVKWTDALIKANTNKNYIAGEAVTGQPIKLKSHTDG  
TFEIKGLAYAVDANAEGTAVTYKLKETKAPEGYVIPDKEIEFTVSQTSYNTKPTDITVDSADATPDTI  
KNNKRPS

**40** Applicants have identified a particularly immunogenic fragment of the GBS 80 protein. This immunogenic fragment is located towards the N-terminus of the protein and is underlined in the GBS 80 SEQ ID NO: 2 sequence below. The underlined fragment is set forth below as SEQ ID NO: 8.

**SEQ ID NO: 2**

MKLSKKLLFSAAVLTMVAGSTVEPVAQFATGMSIVRAAEVSQERPAKTTVNIYKLQADSYKSEITSNG  
 GIENKDGEVISNYAKLGDNVKGLQGVQFKRYKVKTDISVDELKKLTTVEAADAKVGTTILEEGVSLPQK  
 5 TNAQGLVVDALDSKSNVRYLYVEDLKNSPSNITKAYAVPFVLELPVANSTGTGFLSEINIYPKNVVTD  
 EPKTDKDVKKLGQDDAGYTIGEEFKWFLKSTIPANLGDYEKFEITDKFADGLTYKSVGKIKIGSKTLN  
 RDEHYTIDEPTVDNQNTLKITFKPEKFKEIAELLKGMTLVKNQDALDKATANTDDAAFLEIPVASTIN  
 EKAVLGKAIENTFELQYDHTPDKADNPKPSNPPRKPEVHTGGKRFVKKDSTETQTLGGAEFDLLASDG  
 TAVKWTDALIKANTNKNYIAGEAVTGQPIKLKSHDTGTFEIKGLAYAVDANAEGTAVTYKLKETKAPE  
 10 GYVIPDKEIEFTVSQTSYNTKPTDITVDSADATPDTIKNNKRPSIPNTGGIGTAIFVAIGAAMAFVAV  
 KGMKRRTKDN

**SEQ ID NO: 8**

AEVSQERPAKTTVNIYKLQADSYKSEITSNGGIENKDGEVISNYAKLGDNVKGLQGVQFKRYKVKTDI  
 15 SVDELKKLTTVEAADAKVGTTILEEGVSLPQKTNAQGLVVDALDSKSNVRYLYVEDLKNSPSNITKAYA  
 VPFVLELPVANSTGTGFLSEINIYPKNVVTDEPKTDKDVKKLGQDDAGYTIGEEFKWFLKSTIPANL  
 DYEKFEITDKFADGLTYKSVGKIKIGSKTLNRDEHYTIDEPTVDNQNTLKITFKPEKFKEIAELLKG

The immunogenicity of the protein encoded by SEQ ID NO: 7 was compared against PBS,  
 20 GBS whole cell, GBS 80 (full length) and another fragment of GBS 80, located closer to the C-  
 terminus of the peptide (SEQ ID NO: 9, below).

**SEQ ID NO: 9**

MTLVKNQDALDKATANTDDAAFLEIPVASTINEKAVLGKAIENTFELQYDHTPDKADNPKPSNPPRKPEVHTGGK  
 25 RFVKKDSTETQTLGGAEFDLLASDGTAVKWTDALIKANTNKNYIAGEAVTGQPIKLKSHDTGTFEIKGLAYAVDA  
 NAEGTAVTYKLKETKAPEGYVIPDKEIEFTVSQTSYNTKPTDITVDSADATPDTIKNNKRPS

Both an Active Maternal Immunization Assay and a Passive Maternal Immunization Assay  
 were conducted on this collection of proteins.

As used herein, an Active Maternal Immunization assay refers to an *in vivo* protection assay  
 30 where female mice are immunized with the test antigen composition. The female mice are then bred  
 and their pups are challenged with a lethal dose of GBS. Serum titers of the female mice during the  
 immunization schedule are measured as well as the survival time of the pups after challenge.

Specifically, the Active Maternal Immunization assays referred to herein used groups of four  
 CD-1 female mice (Charles River Laboratories, Calco Italy). These mice were immunized  
 35 intraperitoneally with the selected proteins in Freund's adjuvant at days 1, 21 and 35, prior to  
 breeding. 6-8 weeks old mice received 20 µg protein/dose when immunized with a single  
 antigen, 30-45 µg protein/dose (15 µg each antigen) when immunized with combination of  
 antigens. The immune response of the dams was monitored by using serum samples taken on day  
 0 and 49. The female mice were bred 2-7 days after the last immunization (at approximately t=  
 40 36 – 37), and typically had a gestation period of 21 days. Within 48 hours of birth, the pups were  
 challenged via I.P. with GBS in a dose approximately equal to an amount which would be  
 sufficient to kill 70 – 90 % of unimmunized pups (as determined by empirical data gathered from



PBS control groups). The GBS challenge dose is preferably administered in 50 $\mu$ l of THB medium. Preferably, the pup challenge takes place at 56 to 61 days after the first immunization. The challenge inocula were prepared starting from frozen cultures diluted to the appropriate concentration with THB prior to use. Survival of pups was monitored for 5 days after challenge.

As used herein, the Passive Maternal Immunization Assay refers to an *in vivo* protection assay where pregnant mice are passively immunized by injecting rabbit immune sera (or control sera) approximately 2 days before delivery. The pups are then challenged with a lethal dose of GBS.

Specifically, the Passive Maternal Immunization Assay referred to herein used groups of pregnant CD1 mice which were passively immunized by injecting 1 ml of rabbit immune sera or control sera via I.P., 2 days before delivery. Newborn mice (24-48 hrs after birth) are challenged via I.P. with a 70 - 90% lethal dose of GBS serotype III COH1. The challenge dose, obtained by diluting a frozen mid log phase culture, was administered in 50 $\mu$ l of THB medium.

For both assays, the number of pups surviving GBS infection was assessed every 12 hrs for 4 days. Statistical significance was estimated by Fisher's exact test.

The results of each assay for immunization with SEQ ID NO: 7, SEQ ID NO: 8, PBS and GBS whole cell are set forth in Tables 1 and 2 below.

<b>TABLE 1: Active Maternal Immunization</b>			
Antigen	Alive/total	%Survival	Fisher's exact test
PBS (neg control)	13/80	16%	
GBS (whole cell)	54/65	83%	P<0.00000001
GBS80 (intact)	62/70	88%	P<0.00000001
GBS80 (fragment) SEQ ID 7	35/64	55%	P=0.0000013
GBS80 (fragment) SEQ ID 8	13/67	19%	P=0.66

<b>Table 2: Passive Maternal Immunization</b>			
Antigen	Alive/total	%Survival	Fisher's exact test
PBS (neg control)	12/42	28%	
GBS (whole cell)	48/52	92%	P<0.00000001
GBS80 (intact)	48/55	87%	P<0.00000001
GBS80 (fragment) SEQ ID 7	45/57	79%	P=0.0000006
GBS80 (fragment) SEQ ID 8	13/54	24%	P=1

As shown in Tables 1 and 2, immunization with the SEQ ID NO: 7 GBS 80 fragment provided a substantially improved survival rate for the challenged pups than the comparison SEQ ID NO: 8 GBS 80 fragment. These results indicate that the SEQ ID NO: 7 GBS 80 fragment may comprise an important immunogenic epitope of GBS 80.

Combinations including GBS 80

The invention includes combinations of two or more GBS antigens wherein the combination includes GBS 80 or a fragment thereof. Applicants have discovered that GBS 80 is particularly suitable for immunization in combination with other GBS antigens and that these antigen combinations provide for a broader coverage among different GBS strains.

Preferably, the combination of GBS antigens consists of three, four, five, six, seven, eight, nine, or ten GBS antigens. Still more preferably, the combination of GBS antigens consists of three, four, or five GBS antigens.

Preferably, the combinations of the invention provide for improved immunogenicity over the immunogenicity of the antigens when administered alone. Improved immunogenicity may be measured, for example, by the Active Maternal Immunization Assay. As discussed above, this assay may be used to measure serum titers of the female mice during the immunization schedule as well as the survival time of the pups after challenge. Preferably, immunization with the immunogenic compositions of the invention yield an increase of at least 2 percentage points (preferably at least 3, 4 or 5 percentage points) in the percent survival of the challenged pups as compared to the percent survival from maternal immunization with a single antigen of the composition when administered alone. Preferably, the increase is at least 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29 or 30 percentage points. Preferably, the GBS combinations of the invention comprising GBS 80 demonstrate an increase in the percent survival as compared to the percent survival from immunization with a non-GBS 80 antigen alone.

According to one embodiment of the invention, combinations of antigens or fusion proteins containing a portion or portions of the antigens will include GBS 80 or a portion thereof in combination with from one to 10 antigens, preferably one to 10 or less antigens. Such other antigens include by way of example and not limitation, GBS 67, GBS 91, GBS 104, GBS 184, GBS 276, GBS 305, GBS 322, GBS 330, GBS 338, GBS 361, GBS 404, GBS 690, and GBS 691. Still other antigens are identified in U.S. Serial Number 10/415,182, filed April 28, 2003, hereby incorporated in its entirety.

Combinations, for example, can include GBS 80, GBS 104, GBS 322, and GBS 276, ; GBS 80, GBS 338, GBS 330; GBS 80, GBS 330, GBS 104; GBS 80, GBS 104, GBS 404; GBS 80, GBS 338, GBS 104; GBS 80, GBS 338, GBS 404; GBS 338, GBS 330, GBS 104; GBS 338, GBS 104, GBS 404; GBS 80, GBS 330, GBS 404; GBS 80, GBS 322, GBS 104; GBS 80, GBS 322, GBS 276; GBS 80, GBS 322, GBS 91; GBS 80, GBS 104, GBS 276; GBS 80, GBS 104, GBS 91; GBS 80, GBS 276, GBS 91; GBS 80, GBS 322, GBS 104; GBS 80, GBS 322, GBS 276; GBS 80, GBS 322, GBS 91; GBS 80, GBS 104, GBS 276; GBS 80, GBS 104, GBS 91; GBS 80, GBS 276, GBS 91; GBS 80, GBS 690, GBS 691; GBS 80, GBS 690, GBS 338; GBS 80, GBS 690, GBS 305; GBS 80, GBS 691,

GBS 305; GBS 80, GBS 338, GBS 305; GBS 80, GBS 338, GBS 361; GBS 80, GBS 305, GBS 361; GBS 80, GBS 184, GBS 691; GBS 80, GBS 691, GBS 338; GBS 80, GBS 104, GBS 276, GBS 322; GBS 80, GBS 104, GBS 67, and GBS 322. Examples of combinations of the invention which demonstrate improved immunogenicity are set forth below. A more detailed description of the GBS antigens referred to in these experiments is set forth following the examples.

**EXAMPLE 1: Active Maternal Immunization Assay of GBS 80 alone vs. in combination**

In this example, the Active Maternal Immunization Assay was used to measure the percent survival of pups challenged with a Type III serotype of GBS (COH1 isolate), at t=56 days. The maternal mice were immunized according to the Active Maternal Immunization Assay schedule discussed above with GBS 80 alone, combinations of GBS antigens (with and without GBS 80), placebo (PBS) or inactivated whole cell GBS isolate as indicated in Table 3 below. In these experiments, the challenge dose for GBS Type III, strain isolate COH1 sufficient to kill 70 – 90 % of unimmunized pups is approximately equal to 10 x LD 50% (where LD 50% is the statistically derived Median Lethal Dose).

Table 3: Active Maternal Immunization Assay of GBS 80 alone vs. in combination

$\alpha$ -GBS	I Challenge t=56 days Type III COH1 10 x LD 50%	
	Alive/treated	Survival %
$\alpha$ -PBS	3/26	11
$\alpha$ -GBS III	9/20	45
80	24/34	70
80+338+330	39/40	97
80+330+104	38/40	95
80+104+404	24/24	100
80+338+104	33/34	97
80+338+404	30/30	100
338+330+104	22/30	73
338+104+404	24/37	65
80+330+404	25/28	89

As shown in Table 3, combinations of GBS antigens which included GBS 80 demonstrated an improved immunogenicity over the use of the antigens alone. For example, immunization with GBS 80 alone yielded a 70% survival rate among the challenged pups. Immunization with combinations of GBS 80 with GBS 338, GBS 330, GBS 104, and GBS 404 yielded 95 to 100% survival rate among the challenged pups. This is an increase of 25 to 30 percentage points.

By comparison, combinations of these antigens which did not include GBS 80 failed to achieve the % survival of GBS 80 alone. For example, immunization with GBS 338, GBS 104 and

GBS 404 yielded a 65% survival rate. Replacement of any one of these antigens with GBS 80 dramatically increased the percent survival rate to between 97 and 100%. This is an increase of 32 to 35 percentage points. (See percent survival rates of GBS 80, 338, 101 (97%); GBS 80, 338, 404 (100%) and GBS 80, 104, 404 (100%)). Similarly, immunization with GBS 338, 330 and 104 yielded a 73% survival rate. Replacement of any one of these antigens with GBS 80 increased the percent survival rate to between 95 – 97%.

These findings indicate that protection from COH1 isolate is increased with use of GBS 80 in combination with other GBS antigens.

**EXAMPLE 2: Active Maternal Immunization Assay of GBS 80, GBS 322, GBS 276, GBS 104 alone vs. in combination**

In this example, the Active Maternal Immunization Assay was used to measure the percent survival of pups challenged with a Type III serotype of GBS (COH1 isolate) at t=56 days. The maternal mice were immunized according to the Active Maternal Immunization Assay schedule discussed above with a single GBS antigen, combinations of GBS antigens with GBS 80, and placebo (PBS) as indicated in Table 4 below.

Table 4: Active Maternal Immunization Assay of GBS 80, GBS 322, GBS 276 or GBS 104 alone vs. in combination with GBS 80

$\alpha$ -GBS	I Challenge t=56 days Type III COH1 10x LD 50%	
	Alive/treated	Survival %
80 + 322 + 104	27/27	100
80 + 322 + 276	35/38	92
80 + 322 + 91	24/24	100
80 + 104 + 276	29/30	97
80 + 104 + 91	36/40	90
80 + 276 + 91	33/40	82
GBS 80	24/30	80
GBS 322	7/40	17
GBS 276	13/37	35
GBS 104	28/38	74
$\alpha$ -PBS	2/27	7

As shown in Table 4, the combinations of the antigens with GBS 80 yielded improved immunogenicity over the use of the antigens alone. For example, immunization with GBS 322 alone yielded a 17 % survival rate among the challenged pups. Immunization with combinations of GBS 322 with GBS 80 and another GBS antigen yielded survival rates of 92 – 100%. As another example, immunization with GBS 104 alone yielded a 74% survival rate. Immunization with combinations of

GBS 104 with GBS 80 and another GBS antigen yielded survival rates of 90 – 100%. As another example, immunization with GBS 276 alone yielded a 35% survival rate. Immunization with combinations of GBS 276 with GBS 80 and another GBS antigen yielded survival rates of 82 – 97%.

Having demonstrated the immunogenicity of the above-described combinations, the duration of the immune response in the mouse model was further analysed. The maternal mice used in the above described Active Maternal Immunization Assay were mated a second time and the resulting pups challenged with a different GBS serotype (Type V, CJB 111 isolate) at a dramatically higher dose (300x LD 50%) at t=91 days. The parameters of this second, much stronger challenge were outside those of the standard Active Maternal Immunization Assay and were meant to probe the limits of the immunological memory generated from the original maternal immunization in the mouse model. Indication of immunological memory in this model under these conditions is thought to be significant. As shown in Table 5, even under these extreme conditions, increased survival rates were generally achieved, particularly for the combination comprising GBS 80, GBS 322 and GBS 104. It was surprising to note that the percent survival rate for the combination of GBS 80, GBS 233 and GBS 104 was 100% for both the first and second challenges.

Table 5: Second generation pups challenged with higher dose of different strain

$\alpha$ -GBS	II Challenge t=91 days Type V CJB111 300x LD 50%	
	Alive/treated	Survival %
80 + 322 + 104	20/20	100
80 + 322 + 276	32/37	86
80 + 322 + 91	27/30	90
80 + 104 + 276	22/37	59
80 + 104 + 91	36/39	92
80 + 276 + 91	23/28	82
GBS 80	13/30	43
GBS 322	25/30	83
GBS 276	18/40	45
GBS 104	21/39	54
$\alpha$ -PBS	9/36	25

**EXAMPLE 3: Active Maternal Immunization Assay of combinations of GBS 80 with GBS 690, GBS 691, GBS 338, GBS 305, GBS 361 and GBS 184**

In this example additional combinations of GBS antigens were used in the Active Maternal Immunization Assay, again with a GBS Type III COH1 isolate challenge. The maternal mice were immunized according to the Active Maternal Immunization Assay schedule described above with the combinations of GBS antigens set forth in Table 6 below.

Table 6: Active Maternal Immunization Assay using combinations of GBS 80 with GBS 690, GBS 691, GBS 338, GBS 305, GBS 361 and GBS 184

$\alpha$ -GBS	I Challenge t=56 days Type III COH1 10x LD 50%	
	Alive/treated	Survival %
80 + 690 + 691	26/29	90
80 + 690 + 338	35/40	87
80 + 690 + 305	34/35	97
80 + 691 + 305	37/40	92
80 + 338 + 305	25/30	83
80 + 338 + 361	26/30	87
80 + 305 + 361	23/30	77
80 + 184 + 691	32/39	82
$\alpha$ -PBS	10/40	25

5           The maternal mice in this model were also mated a second time and the resulting pups  
 challenged with the same GBS isolate at a dramatically higher dose (100x LD 50%) at t=84 days. As  
 in the example above, the parameters of this second, much stronger challenge were outside those of  
 the standard Active Maternal Immunization Assay and were meant to probe the limits of the  
 immunological memory generated from the original maternal immunization in the mouse model. As  
 10 shown in Table 7, even under these extreme conditions, some of the survival rates remained at or  
 above 70%. Surprisingly, the percent survival rates for the combination of GBS 80, GBS 184 and  
 GBS 691 actually increased.

Table 7: Second generation pups challenged with higher dose

$\alpha$ -GBS	II Challenge t=84 days Type III COH1 100x LD 50%	
	Alive/treated	Survival %
80 + 690 + 691	19/39	49
80 + 690 + 338	21/30	70
80 + 690 + 305	23/40	57
80 + 691 + 305	22/30	73
80 + 338 + 305	18/30	60
80 + 338 + 361	25/40	62
80 + 305 + 361	21/30	70
80 + 184 + 691	35/40	87
$\alpha$ -PBS	4/20	20

**EXAMPLE 4: Active Maternal Immunization Assay using combinations of GBS 80 with GBS 690, GBS 691, GBS 338, GBS 305, and GBS 361**

In this example additional combinations of GBS antigens were used in the Active Maternal Immunization Assay, this time with a GBS Type V, CJB111 isolate challenge. In these experiments, the challenge dose for the GBS Type V, CJB111 isolate sufficient to kill 70 – 90% of unimmunized pups is approximately equal to 60 x LD 50% (where LD 50% is the statistically derived Median Lethal Dose). The maternal mice were immunized according to the Active Maternal Immunization Assay schedule described above with the combinations of GBS antigens set forth in Table 8 below. As shown in Table 8, in this particular challenge study with this specific Type V strain isolate, the survival rates for all of the combinations achieved at least 70%.

Table 8: Active Maternal Immunization Assay using combinations of GBS 80 with GBS 690, GBS 691, GBS 338, GBS 305 and GBS 361

$\alpha$ -GBS	I Challenge t=56 days Type V CJB111 60x LD 50%	
	Alive/treated	Survival %
80 + 690 + 691	24/30	80
80 + 690 + 338	11/17	70
80 + 691 + 338	7/10	70
80 + 691 + 305	21/30	70
80 + 338 + 305	26/30	87
80 + 338 + 361	26/30	87
80 + 305 + 361	28/30	93
GBS 80	21/30	70
$\alpha$ -PBS	5/18	28

The maternal mice in this model were also mated a second time and the resulting pups challenged with the same GBS isolate at a dramatically higher dose (600x LD 50%) at t=84 days. As in the example above, the parameters of this second, much stronger challenge were outside those of the standard Active Maternal Immunization Assay and were meant to probe the limits of the immunological memory generated from the original maternal immunization in the mouse model. As shown in Table 9, even under these extreme conditions, some of the survival rates remained above 70%. Surprisingly, the percent survival for two of the antigen groups actually increased (GBS 80, GBS 690 and GBS 338) and (GBS 80, GBS 691 and GBS 338).

Table 9: Second generation pups challenged with higher dose

$\alpha$ -GBS	II Challenge t=84 days	
	Type V CJB111 600x LD 50%	
	Alive/treated	Survival %
80 + 690 + 691	27/37	73
80 + 690 + 338	15/20	75
80 + 691 + 338	27/30	90
80 + 691 + 305	23/40	57
80 + 338 + 305	12/20	60
80 + 338 + 361	24/30	80
80 + 305 + 361	24/30	80
GBS 80	24/30	80
$\alpha$ -PBS	ND	ND

**EXAMPLE 5: Active Maternal Immunization Assay using combinations of GBS 80 with GBS 104, GBS 276, and GBS 322**

In this example an additional combination of GBS antigens was used in the Active Maternal Immunization Assay, this time with an isolate challenge of different GBS strains. In these experiments, the challenge dose for the different GBS strains was sufficient to kill 60 – 90% of unimmunized pups and is equal to 10 x LD 50% (where LD 50% is the statistically derived Median Lethal Dose). The maternal mice were immunized according to the Active Maternal Immunization Assay schedule described above with the combination of GBS 80 antigen with GBS 104, GBS 276, and GBS 322 antigens in the GBS strains set forth in Table 10 below. Survival % was observed with the GBS combination with two different adjuvants, Alum and Freund's. As shown in Tables 10 and 11, in this particular challenge study, the survival rates for the combination in all of the GBS strains achieved up to 96%.



Table 10: Active Maternal Immunization Assay using combinations of GBS 80 with GBS 104, GBS 276, and GBS 322 – Alum adjuvant

<b>ALUM</b>					
<b>Mix=322+80+104+276</b>				<b>PBS</b>	
<b>GBS strains</b>	<b>Type</b>	<b>Alive/treated</b>	<b>Survival %</b>	<b>Alive/treated</b>	<b>Survival %</b>
JM9130013	VIII	32/36	89	18/46	40
CJB111	V	118/145	81	21/110	19
COH1	III	96/115	83	22/104	21
M781	III	42/52	81	18/48	38
2603	V	79/145	54	28/128	22
18RS21	II	86/186	46	24/131	18
DK21	II	31/140	22	28/118	24
7357b –	Ib	25/88	28	25/106	23
A909	Ia	4/40	10	9/60	15
090	Ia	2/31	6	4/53	7
SMO53	VII	17/54	31	4/39	10

5

Table 11: Active Maternal Immunization Assay using combinations of GBS 80 with GBS 104, GBS 276, and GBS 322 – Freund adjuvant

<b>Freund</b>					
<b>Mix=322+80+104+276</b>				<b>PBS</b>	
<b>GBS strains</b>	<b>Type</b>	<b>Alive/treated</b>	<b>Survival %</b>	<b>Alive/treated</b>	<b>Survival %</b>
JM9130013	VIII	nd	nd	nd	nd
CJB111	V	47/49	96	12/46	26
COH1	III	47/50	94	12/50	24
M781	III	33/50	66	6/50	12
2603	V	28/30	93	8/48	17
18RS21	II	31/78	40	10/46	22
DK21	II	37/68	54	15/60	25
H36B	Ib	8/38	21	5/60	8
7357b –	Ib	29/50	58	5/50	10
A909	Ia	18/49	37	6/49	12

Accordingly, the invention therefore includes compositions comprising combinations of two or more GBS antigens, wherein the combination includes GBS 80 or a fragment thereof or a polypeptide sequence having sequence identity thereto.

10

In one embodiment, the combination may consist of two to thirteen GBS antigens, including GBS 80. As an example, the combination may contain GBS 80 and other GBS antigens selected from the group consisting of GBS 80, GBS 91, GBS 104, GBS 184, GBS 276, GBS 305, GBS 322, GBS 330, GBS 338, GBS 361, GBS 404, GBS 690, and GBS 691. Preferably, the combination includes

GBS 80 in combination with one or more of GBS 104 and GBS 322. For example, the combination may include GBS 80, GBS 104, GBS 322 and GBS 67.

Instead of the full length antigen, the combination may comprise an immunogenic fragment of the selected GBS antigen and/or a polypeptide sequence having sequence identity to the selected antigen.

Preferably, the combination of GBS antigens consists of three, four, five, six, seven, eight, nine, or ten GBS antigens. Still more preferably, the combination of GBS antigens consists of three, four, or five GBS antigens.

Details of examples of GBS antigens for use in combination with GBS 80 are set forth below.

#### GBS 91

GBS 91 refers to a GBS C3 binding polypeptide. Nucleotide and amino acid sequences of GBS 91 sequenced from serotype V isolated strain 2603 V/R are set forth in Ref. 2 as SEQ ID 8937 and SEQ ID 8938. These sequences are set forth below as SEQ ID NOS 10 and 11:

#### **SEQ ID NO. 10**

ATGAAAAAAGGACAAGTAAATGATACTAAGCAATCTTACTCTCTACGTAAATATAAATTTGGTTTAGC  
ATCAGTAATTTTAGGGTCATTCATAATGGTCACAAGTCCTGTTTTGCGGATCAAACATACATCGGTTTC  
AAGTTAATAATCAGACAGGCACTAGTGTGGATGCTAATAATTCTTCCAATGAGACAAGTGCCTCAAGT  
GTGATTACTTCCAATAATGATAGTGTTCAAGCGTCTGATAAAGTTGTAAATAGTCAAAATACGGCAAC  
AAAGGACATTACTACTCCTTTAGTAGAGACAAAGCCAATGGTGAAAAAACATTACCTGAACAAGGGA  
ATTATGTTTATAGCAAAGAAACCGAGGTGAAAAATACACCTTCAAATCAGCCCCAGTAGCTTTCTAT  
GCAAAGAAAGGTGATAAAGTTTCTATGACCAAGTATTTAATAAAGATAATGTGAAATGGATTTCATA  
TAAGTCTTTTTGTGGCGTACGTCGATACGCAGCTATTGAGTCACTAGATCCATCAGGAGGTTTCAGAGA  
CTAAAGCACCTACTCCTGTAACAAATTCAGGAAGCAATAATCAAGAGAAAAATAGCAACGCAAGGAAAT  
TATACATTTTACATAAAGTAGAAGTAAAAAATGAAGCTAAGGTAGCGAGTCCAACCTCAATTTACATT  
GGACAAAGGAGACAGAATTTTTTACGACCAATACTAATCTATTGAAGGAAATCAGTGGTTATCTTATA  
AATCATTTCAATGGTGTTCGTCGTTTTGTTTTGCTAGGTAAAGCATCTTCAGTAGAAAAAACTGAAGAT  
AAAGAAAAAGTGTCTCCTCAACCACAAGCCCGTATTACTAAAACCTGGTAGACTGACTATTTCTAACGA  
AACAACCTACAGGTTTTGATATTTAATTACGAATATTAAAGATGATAACGGTATCGCTGCTGTTAAGG  
TACCGGTTTTGGACTGAACAAGGAGGGCAAGATGATATTAAATGGTATACAGCTGTAACCTACTGGGGAT  
GGCAACTACAAAGTAGCTGTATCATTTGCTGACCATAAGAATGAGAAGGGTCTTTATAATATTCATTT  
ATACTACCAAGAAGCTAGTGGGACACTTGTAGGTGTAACAGGAACTAAAGTGACAGTAGCTGGAACCTA  
ATTCTTCTCAAGAACCTATTGAAAATGGTTTAGCAAAGACTGGTGTTTATAATATTATCGGAAGTACT  
GAAGTAAAAAATGAAGCTAAAATATCAAGTCAGACCCAATTTACTTTAGAAAAAGGTGACAAAATAAA  
TTATGATCAAGTATTGACAGCAGATGGTTACCAGTGGATTTCTTACAAATCTTATAGTGGTGTTCGTC  
GCTATATTCCTGTGAAAAAGCTAACTACAAGTAGTGAAAAAGCGAAAGATGAGGCGACTAAACCGACT  
AGTTATCCCAACTTACCTAAAACAGGTACCTATACATTTACTAAAACCTGTAGATGTGAAAAGTCAACC  
TAAAGTATCAAGTCCAGTGGAAATTTAATTTTCAAAGGGTGAAAAAATACATTATGATCAAGTGTTAG  
TAGTAGATGGTCATCAGTGGATTTTCATACAAGAGTTATTCCGGTATTCGTCGCTATATTGAAATT

#### **SEQ ID NO. 11**

MKKGQVNDTKQSYSLRKYKFGLASVILGSFIMVTSVPVFADQTTSVQVNNQTGTSVDANNSSNETSASS  
VITSNNDSVQASDKVVNSQNTATKDIITPLVETKPMVEKTLPEQGNVYVSKETEVENKTPSKSAPVAFY  
AKKGDVKFYDQVFENKDNVWISYKSFVRRYAAIESLDPSSGSETKAPTPTVNSGNNQEKIATQGN  
YTFSHKVEVKNEAKVASPTQFTLDKGDRIFYDQILTIENQWLSYKSFNGVRRFVLLGKASSVEKTED  
KEKVSPQPQARITKTGRLTISNETTTGFDILITNIKDDNGIAAVKVPVWTEQGGQDDIKWYTAVTTGD

GNYKVAVSFADHKNEKGLYNIHLYYQEASGTLVGVGTGKVTVAGTNSSQEPIENGLAKTGVYNIIGST  
 EVKNEAKISSQTQFTLEKGDKINYDQVLTADGYQWISYKSYSGVRRYIPVKKLTTSSSEKAKDEATKPT  
 SYPNLPKTGT~~YTF~~TKTVDVKSQPKVSSPVEFNFQKGEKIHYDQVLVVDGHWISYKSYSGIRRYIEI

- 5 GBS 91 contains an N-terminal leader or signal sequence region which is indicated by the underlined sequence at the beginning of SEQ ID NO: 11 above. In one embodiment, one or more amino acids from this leader or signal sequence region of GBS 91 are removed. An example of such a GBS 91 fragment is set forth below as SEQ ID NO: 12.

10 **SEQ ID NO: 12**

DQTTSVQVNNQTGTSVDANNSSNETSASSVITSNNDSVQASDKVVNSQNTATKDITTPLVETKPMVEK  
 TLPEQGNVYVSKETEVKNTPSKSAPVAFYAKKGDKVFYDQVFNKDNVKWISYKSYSGVRRYAAIESLD  
 PSGGSETKAPTPVTNSGSNNQEKIATQGNITFSHKVEVKNEAKVASPTQFTLDKGDRIFYDQILITIEG  
 15 NQWLSYKSFNGVRRFVLLGKASSVEKTEDKEKVSPQPQARITKTGRLTISNETTTGFDILITNIKDDN  
 GIAAVKVPVWTEQGGQDDIKWYTAVTTGDGNYKVAVSFADHKNEKGLYNIHLYYQEASGTLVGVGTGK  
 VTVAGTNSSQEPIENGLAKTGVYNIIGSTEVKNEAKISSQTQFTLEKGDKINYDQVLTADGYQWISYK  
 SYSGVRRYIPVKKLTTSSSEKAKDEATKPTSYPNLPKTGT~~YTF~~TKTVDVKSQPKVSSPVEFNFQKGEKI  
 HYDQVLVVDGHWISYKSYSGIRRYIEI

- 20 GBS 91 contains a C-terminal transmembrane region which may be located within the underlined region near the end of SEQ ID NO: 11 above. In one embodiment, one or more amino acids from the transmembrane and cytoplasmic regions are removed. An example of such a GBS 91 fragment is set forth below as SEQ ID NO: 13.

25 **SEQ ID NO: 13**

MKKGQVNDTKQSYSLRKYKFGLASVILGSFIMVTSPVFADQTTSVQVNNQTGTSVDANNSSNETSASS  
 VITSNNDSVQASDKVVNSQNTATKDITTPLVETKPMVEKTLPEQGNVYVSKETEVKNTPSKSAPVAFY  
 AKKGDKVFYDQVFNKDNVKWISYKSYSGVRRYAAIESLDPSGGSETKAPTPVTNSGSNNQEKIATQGN  
 30 YTFSHKVEVKNEAKVASPTQFTLDKGDRIFYDQILITIEGNQWLSYKSFNGVRRFVLLGKASSVEKTED  
 KEKVSPQPQARITKTGRLTISNETTTGFDILITNIKDDNGIAAVKVPVWTEQGGQDDIKWYTAVTTGD  
 GNYKVAVSFADHKNEKGLYNIHLYYQEASGTLVGVGTGKVTVAGTNSSQEPIENGLAKTGVYNIIGST  
 EVKNEAKISSQTQFTLEKGDKINYDQVLTADGYQWISYKSYSGVRRYIPVKKLTTSSSEKAKDEATKPT  
 SYPNLPKTG

- 35 GBS 91 contains an amino acid motif indicative of a cell wall anchor: **SEQ ID NO: 14**  
 LTKTG (shown in italics in SEQ ID NO: 11 above). In one embodiment, both the transmembrane domain and the cell wall anchor motif are removed from GBS 91. An example of such a GBS 91 fragment is set forth below as SEQ ID NO: 15.

40 **SEQ ID NO: 15**

MKKGQVNDTKQSYSLRKYKFGLASVILGSFIMVTSPVFADQTTSVQVNNQTGTSVDANNSSNETSASS  
 VITSNNDSVQASDKVVNSQNTATKDITTPLVETKPMVEKTLPEQGNVYVSKETEVKNTPSKSAPVAFY  
 AKKGDKVFYDQVFNKDNVKWISYKSYSGVRRYAAIESLDPSGGSETKAPTPVTNSGSNNQEKIATQGN  
 45 YTFSHKVEVKNEAKVASPTQFTLDKGDRIFYDQILITIEGNQWLSYKSFNGVRRFVLLGKASSVEKTED  
 KEKVSPQPQARITKTGRLTISNETTTGFDILITNIKDDNGIAAVKVPVWTEQGGQDDIKWYTAVTTGD  
 GNYKVAVSFADHKNEKGLYNIHLYYQEASGTLVGVGTGKVTVAGTNSSQEPIENGLAKTGVYNIIGST

EVKNEAKISSQTQFTLEKGDKINYDQVLTADGYQWISYKSYSGVRRYIPVKKLTTSSSEKAKDEATKPT  
SYPN

In one embodiment, one or more amino acids from the leader or signal sequence region and  
5 one or more amino acids from the transmembrane and cytoplasmic regions are removed from the GBS  
91 sequence. An example of such a GBS 91 fragment is set forth below as SEQ ID NO: 16.

**SEQ ID NO: 16**

DQTTSVQVNNQTGTSVDANSSNETSASSVITSNNDSVQASDKVVNSQNTATKDITTPLVETKPMVEK  
10 TLPEQGNVYVSKETEVKNTPSKSAPVAFYAKKGDKVFYDQVFNKDNVWKWISYKSFVRRYAAIESLD  
PSGGSETKAPTPTNSGSNNQEKIATQGNVYFVSHKVEVKNEAKVASPTQFTLDKGDRIFYDQILTIEG  
NQWLSYKSFNGVRRFVLLGKASSVEKTEDKEKVSPQPQARITKTGRLTISNETTTGFDILITNIKDDN  
GIAAVKVPVWTEQGGQDDIKWYTAVTTGDGNYKVAVSFADHKNEKGLYNIHLYYQEASGTLVGVGTGK  
15 VTVAGTNSSQEPIENGLAKTGVYNIIGSTEVEKNEAKISSQTQFTLEKGDKINYDQVLTADGYQWISYK  
SYSGVRRYIPVKKLTTSSSEKAKDEATKPTSYPNLPKTG

In another embodiment, the leader or signal sequence region, the transmembrane and  
cytoplasmic regions, and the cell wall anchor motif are all removed from the GBS 91 sequence. An  
example of such a GBS 91 fragment is set forth below as SEQ ID NO: 17.

**SEQ ID NO: 17**

DQTTSVQVNNQTGTSVDANSSNETSASSVITSNNDSVQASDKVVNSQNTATKDITTPLVETKPMVEK  
TLPEQGNVYVSKETEVKNTPSKSAPVAFYAKKGDKVFYDQVFNKDNVWKWISYKSFVRRYAAIESLD  
25 PSGGSETKAPTPTNSGSNNQEKIATQGNVYFVSHKVEVKNEAKVASPTQFTLDKGDRIFYDQILTIEG  
NQWLSYKSFNGVRRFVLLGKASSVEKTEDKEKVSPQPQARITKTGRLTISNETTTGFDILITNIKDDN  
GIAAVKVPVWTEQGGQDDIKWYTAVTTGDGNYKVAVSFADHKNEKGLYNIHLYYQEASGTLVGVGTGK  
VTVAGTNSSQEPIENGLAKTGVYNIIGSTEVEKNEAKISSQTQFTLEKGDKINYDQVLTADGYQWISYK  
SYSGVRRYIPVKKLTTSSSEKAKDEATKPTSYPN

Further information regarding GBS 91 can be found in WO 01/25440 (C3 binding  
polypeptide), WO 01/32882 (ID-65), WO 02/31156 (BVH) and Reinscheid et al., *Microbiology*  
(2002) 148: 3245-3254 (*bsp* gene), each of which are incorporated herein by reference in their  
entirety.

**GBS 104**

GBS 104 refers to a putative cell wall surface anchor family protein. It has been referred to as  
emaA protein. Nucleotide and amino acid sequences of GBS 104 sequenced from serotype V isolated  
strain 2603 V/R are set forth in Ref. 2 as SEQ ID 8777 and SEQ ID 8778. These sequences are set  
forth below as SEQ ID NOS 18 and 19:

**SEQ ID NO. 18**

ATGAAAAAGAGACAAAAAATATGGAGAGGGTTATCAGTTACTTTACTAATCCTGTCCCAAATTCATT  
 TGGTATATTGGTACAAGGTGAAACCCAAGATACCAATCAAGCACTTGGAAAAGTAATTGTTAAAAAAA  
 5 CGGGAGACAATGCTACACCATTAGGCAAAGCGACTTTTGTGTTAAAAAATGACAATGATAAGTCAGAA  
 ACAAGTCACGAAACGGTAGAGGGTCTGGAGAAGCAACCTTTGAAAACATAAAACCTGGAGACTACAC  
 ATTAAGAGAAGAAACAGCACCAATTGGTTATAAAAAAACTGATAAAACCTGGAAAGTTAAAGTTGCAG  
 ATAACGGAGCAACAATAATCGAGGGTATGGATGCAGATAAAGCAGAGAAACGAAAAGAAGTTTGAAT  
 10 GCCCAATATCCAAAATCAGCTATTTATGAGGATACAAAAGAAAATTACCCATTAGTTAATGTAGAGGG  
 TTCCAAAGTTGGTGAACAATACAAAGCATTGAATCCAATAAATGGAAAAGATGGTCTGAAGAGAGATTG  
 CTGAAGGTTGGTTATCAAAAAAATTACAGGGGTCAATGATCTCGATAAGAATAAATATAAAATTGAA  
 TTAAGTGTGAGGGTAAACCACTGTTGAAACGAAAGAACTTAATCAACCACTAGATGTCGTTGTGCT  
 ATTAGATAAATCAAATAGTATGAATAATGAAAGAGCCAATAATTCTCAAAGAGCATTTAAAGCTGGGG  
 15 AAGCAGTTGAAAAGCTGATTGATAAAATTACATCAAATAAAGACAATAGAGTAGCTCTTGTGACATAT  
 GCCTCAACCATTTTTGATGGTACTGAAGCGACCGTATCAAAGGGAGTTGCCGATCAAATGGTAAAGC  
 GCTGAATGATAGTGTATCATGGGATTATCATAAACTACTTTTACAGCAACTACACATAATTACAGTT  
 ATTTAAATTTAACAAATGATGCTAACGAAGTTAATATTCTAAAGTCAAGAATTCCAAGGAAGCGGAG  
 CATATAAATGGGGATCGCACGCTCTATCAATTTGGTGCGACATTTACTCAAAGCTCTAATGAAAGC  
 20 AAATGAAATTTTAGAGACACAAAGTTCTAATGCTAGAAAAAACTTATTTTTTACGTAAGTATGGTG  
 TCCCTACGATGTCTTATGCCATAAATTTAATCCTTATATATCAACATCTTACCAAAACCAGTTTAAAT  
 TCTTTTTTAAATAAAATACCAGATAGAAGTGGTATTTCTCCAAGAGGATTTTATAATCAATGGTGATGA  
 TTATCAAATAGTAAAGGAGATGGAGAGAGTTTTAACTGTTTTCGGATAGAAAAGTTCTGTTACTG  
 GAGGAACGACACAAGCAGCTTATCGAGTACCGCAAAATCAACTCTCTGTAATGAGTAATGAGGGATAT  
 25 GCAATTAATAGTGGATATATTTATCTCTATTGGAGAGATTACAACCTGGGTCTATCCATTTGATCCTAA  
 GACAAAGAAAGTTTCTGCAACGAAACAAATCAAACTCATGGTGAGCCAACAACATTATACTTTAATG  
 GAAATATAAGACCTAAAGGTTATGACATTTTTACTGTTGGGATTTGGTGTAACCGGAGATCCTGGTGCA  
 ACTCCTCTTGAAGCTGAGAAATTTATGCAATCAATATCAAGTAAACAGAAAATTATACTAATGTTGA  
 TGATACAAATAAAATTTATGATGAGCTAAATAAATACTTTAAACAATTTGTTGAGGAAAACATTCTA  
 TTGTTGATGGAATGTGACTGATCCTATGGGAGAGATGATTGAATTCCAATTAATAAATGGTCAAAGT  
 30 TTTACATGATGATTACGTTTTGGTTGGAATGATGGCAGTCAATTAATAAATGGTGTGGCTCTTGG  
 TGGACCAACAGTGATGGGGGAATTTTAAAGATGTTACAGTGACTTATGATAAGACATCTCAAACCA  
 TCAAATCAATCATTTGAACCTAGGAAGTGACAAAAAGTAGTTCTTACCTATGATGTACGTTTAAAA  
 GATAACTATATAAGTAACAAATTTTACAATAACAAATAATCGTACAACGCTAAGTCCGAAGAGTGAAAA  
 AGAACCATAACTATTCTGTGATTTCCCAATTTCCCAAAATTCGTGATGTTCTGTGAGTTCCGGTACTAA  
 35 CCATCAGTAATCAGAAGAAAATGGGTGAGGTTGAATTTATTAAAGTTAATAAAGACAAACATTAGAA  
 TCGCTTTTGGGAGCTAAGTTTCAACTTCAGATAGAAAAAGATTTTCTGGGTATAAGCAATTTGTTCC  
 AGAGGGAAGTGATGTTACAACAAAGAATGATGGTAAAATTTATTTTAAAGCACTTCAAGATGGTAACT  
 ATAAATTATATGAAATTTCAAGTCCAGATGGCTATATAGAGGTTAAAACGAAACCTGTTGTGACATTT  
 ACAATTCAAATGGAGAAGTTACGAACCTGAAAGCAGATCCAAATGCTAATAAAAAATCAAATCGGGTA  
 40 TCTTGAAGGAAATGGTAAACATCTTATTACCAACACTCCCAAACGCCACCAGGTGTTTTTCTTAAAA  
 CAGGGGGAATTTGGTACAATTGTCTATATATTAGTTGGTTCTACTTTTATGATACTTACCATTTGTTCT  
 TTCCGTCGTAAACAATTG

**SEQ ID NO. 19**

45 MKKRQKIWRGLSVTLILLISQIPFGILVQGETQDTNQLGKVIIVKKTGDNATPLGKATFVLKNDNDKSE  
 TSHEETVEGSGEATFENIKPGDYTLREETAPIGYKKTDKTWKVKVADNGATIEGMDADKAERKEVLN  
 AQYPKSAIYEDTKENYPLVNVEGSKVGEQYKALNPINGKDGREIAEGWLSKKITGVNDLDKNKYKIE  
 LTVEGKTTVETKELNQPLDVVLLDNSNSMNNERANNSQRALKAGEAVEKLIDKITSNKDNRVALVTY  
 50 ASTIFDGTEATVSKGVADQNGKALNDSVSWDYHKTTFTATTHNYSYLNLTNDANEVNILKSRIPEAE  
 HINGDRITLYQFGATFTQKALMKANEILETQSSNARKKLIHFVTDGVPMTSYAINFPYISTSYQNQFN  
 SFLNKIPDRSGILQEDFIINGDDYQIVKGDGESFKLFSDRKVPVTGGTTQAAYRVPQNQLSVMSNEGY  
 AINSYIYLYWRDYNWVYFPDPKTKKVSATKQIKTHGEPTTLYFNGNIRPKGYDIFTVGIGVNGDPGA  
 TPLEAEKFMQSISSKTENYTNVDDTNKIYDELNKYFKTIVEEKHSIVDGNVTDPMGEMIEFQLKNGQS

FTHDDYVLVGNDGSQLKNGVALGGPNSDGGILKDVTVTYDKTSQTIKINHLNLGSGQKVVLTYDVRLK  
 DNYISNKFYNTNNRTTTLSPKSEKEPNTIRDFPIPKIRDVREFPVLTISNQKKMGEVEFIKVNKDKHSE  
 SLLGAKFQLQIEKDFSGYKQFVPEGSDVTTKNDGKIYFKALQDGNYKLYEISSPDGYIEVKTKPVVTF  
 TIQNGEVTNLKADPNANKNQIGYLEGNGKHLITNTPKRPPGVFPKTGGIGTIVYILVGSTFMILTICS  
FRRKQL

GBS 104 contains an N-terminal leader or signal sequence region which is indicated by the underlined sequence at the beginning of SEQ ID NO 19 above. In one embodiment, one or more amino acid sequences from the leader or signal sequence region of GBS 104 are removed. An example of such a GBS 104 fragment is set forth below as SEQ ID NO 20.

**SEQ ID NO 20**

GETQDTNQALGKVIIVKKTGDNATPLGKATFVLKNDNDKSETSHETVEGSGEATFENIKPGDYTLREET  
 APIGYKKTDKTWKVKVADNGATIEGMDADKAERKEVLNAQYPKSAIYEDTKENYPLVNVEGSKVGE  
 QYKALNPINGKDGRRERIEAGWLSKKITGVNDLDKNKYKIELTVEGKTTVETKELNQPLDVVLLDNSN  
 SMNNERANNSQRALKAGEAVEKLIDKITSNKDNVALVTYASTIFDGTEATVSKGVADQNGKALNDSV  
 SWDYHKTTFTATTHNYSYLNLTNDANEVNILKSRIPEAEHINGDRTLYQFGATFTQKALMKANEILE  
 TQSSNARKKLI FHVTDGVPMTSYAINFNPIYSTSYQNQFNSFLNKIPDRSGILQEDFIINGDDYQIVK  
 GDGESFKLFSDRKVPVTGGTTQAAYRVPQNQLSVMSNEGYAINSGYIYLYWRDYNWVYPFDPKTKKVS  
 ATKQIKTHGEPTTLYFNGNIRPKGYDIFTVGIGVNGDPGATPLEAEKFMQSISSKTENYTNVDDTNKI  
 YDELNKYFKTIVEEKHSIVDGNVTDPMGEMIEFQLKNGQSFTHDDYVLVGNDGSQLKNGVALGGPNSD  
 GGILKDVTVTYDKTSQTIKINHLNLGSGQKVVLTYDVRLKDNYISNKFYNTNNRTTTLSPKSEKEPNTI  
 RDFPIPKIRDVREFPVLTISNQKKMGEVEFIKVNKDKHSESLLGAKFQLQIEKDFSGYKQFVPEGSDV  
 TTKNDGKIYFKALQDGNYKLYEISSPDGYIEVKTKPVVTF TIQNGEVTNLKADPNANKNQIGYLEGNG  
 KHLITNTPKRPPGVFPKTGGIGTIVYILVGSTFMILTICSFRRKQL

GBS 104 contains a C-terminal transmembrane and/or cytoplasmic region which is indicated by the underlined region near the end of SEQ ID NO 19 above. In one embodiment, one or more amino acids from the transmembrane or cytoplasmic regions are removed. An example of such a GBS 104 fragment is set forth below as SEQ ID NO 21.

**SEQ ID NO: 21**

MKKRQKIWRGLSVTLILLSQIPFGILVQGETQDTNQALGKVIIVKKTGDNATPLGKATFVLKNDNDKSE  
 TSHETVEGSGEATFENIKPGDYTLREETAPIGYKKTDKTWKVKVADNGATIEGMDADKAERKEVLN  
 AQYPKSAIYEDTKENYPLVNVEGSKVGEQYKALNPINGKDGRRERIEAGWLSKKITGVNDLDKNKYKIE  
 LTVEGKTTVETKELNQPLDVVLLDNSNSMNNERANNSQRALKAGEAVEKLIDKITSNKDNVALVTY  
 ASTIFDGTEATVSKGVADQNGKALNDSVSWDYHKTTFTATTHNYSYLNLTNDANEVNILKSRIPEAE  
 HINGDRTLYQFGATFTQKALMKANEILETQSSNARKKLI FHVTDGVPMTSYAINFNPIYSTSYQNQFN  
 SFLNKIPDRSGILQEDFIINGDDYQIVKGDGESFKLFSDRKVPVTGGTTQAAYRVPQNQLSVMSNEGY  
 AINSGYIYLYWRDYNWVYPFDPKTKKVSATKQIKTHGEPTTLYFNGNIRPKGYDIFTVGIGVNGDPGA  
 TPLEAEKFMQSISSKTENYTNVDDTNKIYDELNKYFKTIVEEKHSIVDGNVTDPMGEMIEFQLKNGQS  
 FTHDDYVLVGNDGSQLKNGVALGGPNSDGGILKDVTVTYDKTSQTIKINHLNLGSGQKVVLTYDVRLK  
 DNYISNKFYNTNNRTTTLSPKSEKEPNTIRDFPIPKIRDVREFPVLTISNQKKMGEVEFIKVNKDKHSE  
 SLLGAKFQLQIEKDFSGYKQFVPEGSDVTTKNDGKIYFKALQDGNYKLYEISSPDGYIEVKTKPVVTF  
 TIQNGEVTNLKADPNANKNQIGYLEGNGKHLITNT

In one embodiment, one or more amino acids from the leader or signal sequence region and one or more amino acids from the transmembrane or cytoplasmic regions are removed. An example of such a GBS 104 fragment is set forth below as SEQ ID NO 22.

5 **SEQ ID NO: 22**

GETQDTNQALGKVIIVKKTGDNATPLGKATFVLKNDNDKSETSHETVEGSGEATFENIKPGDYTLREET  
APIGYKKTDKTWKVKVADNGATIIIEGMDADKAERKEVLNAQYPKSAIYEDTKENYPLVNVESKVG  
QYKALNPINGKDGRRREIAEGWLSKKITGVNDLDKNKYKIELTVEGKTTVETKELNQPLDVVLLDNSN  
10 SMNNERANNSQRALKAGEAVEKLIDKITSNKDNVALVTYASTIFDGTEATVSKGVADQNGKALNDSV  
SWDYHKTTFTATTHNYSYLNLTNDANEVNILKSRIPEAEHINGDRTLYQFGATFTQKALMKANEILE  
TQSSNARKKLI FHVTDGVPTMSYAINFNPIYSTSYQNQFNSFLNKIPDRSGILQEDFIINGDDYQIVK  
GDGESFKLFSDRKVPVTGGTTQAAYRVPQNQLSVMSNEGYAINSGYIYLYWRDYNWVYPFDPKTKKVS  
ATKQIKTHGEPTTLYFNGNIRPKGYDIFTVGIGVNGDPGATPLEAEKFMQSISSTENYTNVDDTNKI  
YDELNKYFKTIVEEKHSIVDGNVTDPMGEMIEFQLKNGQSFTHDDYVLVGNDSQLKNGVALGGPNSD  
15 GGILKDVTVTYDKTSQTIKINHLNLGSGQKVVLTYDVRKLDNYISNKFYNTNNRTTLSPKSEKEPNTI  
RDFPIPKIRDVREFPVLTISNQKKMGEVEFIKVNKDKHSESLGAKFQLQIEKDFSGYKQFVPEGSDV  
TTKNDGKIYFKALQDGNKLYEISSPDGYIEVKT KPVTFTIQNGEVTNLKADPNANKNQIGYLEGNG  
KHLITNT

20 In other embodiments, additional fragments of GBS 104 are provided including an 830 amino acid fragment of GBS 104 of amino acids 28-858, a 359 amino acid fragment of GBS 104 of amino acids 28-387, a 581 amino acid fragment of GBS 104 of amino acids 28-609, or a 740 amino acid fragment of GBS 104 of amino acids 28-768.

25 **GBS 184**

GBS 184 refers to a putative lipoprotein. Nucleotide and amino acid sequences of GBS 184 sequenced from serotype V isolated strain 2603 V/R are set forth in Ref. 2 as SEQ ID 1977 and SEQ ID 1978. These sequences are also set forth below as SEQ ID NOS 23 and 24.

30 **SEQ ID NO: 23**

ATGAAAAAACAACAACTATTACTGCTTATTGGAGGCTTATTAATAATGATAATGATGACAGCATGTAA  
GGATTCAAAAATCCCAGAAAACCGCACAAAGGAAGAGTACCAAGCTGAACAAAATTTTAAACCGTTTT  
TTGAGTTTTTTAGCACAAAAAGATAAAGATTTGAGCAAAATACAAAAATACTTACTATTAGTATCGGAT  
TCAGGTGATGCATTAGATTTAGAATATTTCTATAGTATTCAAGATTTAAAAAAAATAAGGATTTAGG  
35 GAAGTTTGAAACAAGAAAAAGTCAAATAGAAAAGCCGGGTGGCTATAATGAGTTAGAAAATAAAGAGG  
TCCCATTTGAATATTTTAAAAATAATATAGTTTATCCAAAAGGAAAACCGAATATTACATTTGATGAC  
TTTATTATCGGAGCAATGGATACTAAAGAATTAAAAGAATTAAAAAATTAAAAGTAAAAAGTTATTT  
ATTAAAACATCCGGAACTGAGTTGAAAGATATAACATATGAATTGCCGACACAGTCGAAGCTTATTA  
AAAAA

40

**SEQ ID NO: 24**

MKKQKLLLLLIGLLIMIMMTACKDSKIPENRTKEEYQAEQNFKPFFFEFLAQKDKDLSKIQKYLIVSD  
SGDALDLEYFYSIQDLKKNKDLGKFETRKSQIEKPGGYNELENKEVPFEYFKNNIVYPKGKPNITFDD  
FIIGAMDTKELKELKKLVKS YLLKHPETELKDITYELPTQSKLIKK

45

GBS 184 contains a N-terminal leader or signal sequence region which is indicated by the underlined sequence at the beginning of SEQ ID NO 24, above. In one embodiment, one or more amino acids from the leader or signal sequence are removed from GBS 184. An example of such a GBS 184 fragment is set forth below as SEQ ID NO: 25.

**SEQ ID NO: 25**

KDSKIPENRTKEEYQAEQNFKPFFFEFLAQKDKDLSKIQKYL LVS DSGDALDLEYFYISIQDLKKNKDL  
GKFETRKSQIEKPGGYNELENKEVPFEYFKNNIVYPKGKPNITFDDFIIGAMDTKELKELKKLVKSY  
LLKHPETELKDITYELPTQSKLIKK

**GBS 276**

GBS 276 refers to a C5a peptidase. Nucleotide and amino acid sequences of GBS 276 sequenced from serotype V isolated strain 2603 V/R are set forth in Ref. 2 as SEQ ID 8941 and SEQ ID 8942. These sequences are set forth below as SEQ ID NOS 26 and 27:

**SEQ ID NO. 26**

TTGCGTAAAAAACA AAAACTACCATTTGATAAACTTGCCATTGCGCTTATATCTACGAGCATCTTGCT  
CAATGCACAATCAGACATTAAAGCAAATACTGTGACAGAAGACACTCCTGCTACCGAACAAGCCGTAG  
AACC CCCACAACCAATAGCAGTTTCTGAGGAATCACGATCATCAAAGGAACTAAAACCTCACAACT  
CCTAGTGATGTAGGAGAAACAGTAGCAGATGACGCTAATGATCTAGCCCCTCAAGCTCCTGCTAAAAAC  
TGCTGATACACCAGCAACCTCAAAAGCGACTATTAGGGATTTGAACGACCCTTCTCATGTCAAAACCC  
TGCAAGGAAAAAGCAGGCAAGGGAGCTGGGACCGTTGTTGCAGTGATTGATGCTGGTTTTGATAAAAAAT  
CATGAAGCGTGGCGCTTAACAGACAAAACCTAAAGCACGTTACCAATCAAAAGAAAATCTTGAAAAAGC  
TAAAAAAGAGCACGGTATTACCTATGGCGAGTGGGTCAATGATAAGGTTGCTTATTACCACGACTATA  
GTAAAGATGGTAAAAACGCTGTTGATCAAGAACACGGCACACACGCTGTCAGGGATCTTGTCAGGAAAT  
GCTCCATCTGAAATGAAAGAACCTTACCGCCTAGAAGGTGCGATGCCTGAGGCTCAATTGCTTTTGAT  
GCGTGTCGAAATTGTAAATGGACTAGCAGACTATGCTCGTAACTACGCTCAAGCTATCAGAGATGCTG  
TCAACTTGGGAGCTAAGGTGATTAATATGAGCTTTGGTAATGCTGCACTAGCTTACGCCAACCTTCCA  
GACGAAACCAAAAAAGCCTTTGACTATGCCAAATCAAAAGGTGTTAGCATTGTGACCTCAGCTGGTAA  
TGATAGTAGCTTTGGGGGCAAGCCCCGTCTACCTCTAGCAGATCATCCTGATTATGGGGTGGTTGGGA  
CACCTGCAGCGGCAGATTCAACATTGACAGTTGCTTCTTACAGCCCAGATAAACAGCTCACTGAACT  
GCTACGGTCAAAACAGACGATCATCAAGATAAAGAAATGCCTGTTATTTCAACAAACCGTTTTGAGCC  
AAACAAGGCTTACGACTATGCTTATGCTAATCGTGGTACGAAAGAGGATGATTTTAAGGATGTCGAAG  
GTAAGATTGCCCTTATTGAACGTGGCGATATTGATTTCAAAGATAAGATTGCAAACGCTAAAAAAGCT  
GGTGCTGTAGGGGTCTTGATCTATGACAATCAAGACAAGGGCTTCCCGATTGAATTGCCAAATGTTGA  
CCAGATGCCTGCGGCCTTTATCAGTCGAAGAGACGGTCTCTTATTAAAGACAATCCCCCAAAAACCA  
TTACCTTCAATGCGACACCTAAGGTATTGCCAACAGCAAGTGGCACCAAACTAAGCCGCTTCTCAAGC  
TGGGGTCTGACAGCTGACGGCAATATTAAACCGGATATTGCAGCACCCGGCCAAGATATTTTGTCATC  
AGTGGCTAACAACAAGTATGCCAAACTTTCTGGAAGTAGTATGTCTGCACCATGGTAGCGGGTATCA  
TGGGACTGTTGCAAAAGCAATATGAGACACAGTATCCTGATATGACACCATCAGAGCGTCTTGATTTA  
GCTAAGAAAGTATTGATGAGCTCAGCAACTGCCCTATATGATGAAGATGAAAAAGCTTATTTTTCTCC  
TCGCCAACAGGGAGCAGGAGCAGTCGATGCTAAAAAAGCTTCAGCAGCAACGATGTATGTAACAGATA  
AGGACAATACCTCAAGCAAGGTTACCTGAACAATGTTTCTGATAAATTTGAAGTAACAGTAACAGTT  
CACAACAAATCTGATAAACCTCAAGAGTTGTATTACCAAGTAACTGTTCAAACAGATAAAGTAGATGG  
AAAACACTTTGCCCTTGCTCCTAAAGCATTGTATGAGACATCATGGCAAAAAATCACAATTCAGCCA  
ATAGCAGCAACAAGTACCGTTCCAATCGATGCTAGTCGATTTAGCAAGGACTTGCTTGCCCAATG  
AAAAATGGCTATTTCTTAGAAGGTTTGTTCGTTTCAAACAAGATCCTACAAAAGAAGAGCTTATGAG  
CATTCATATATTGGTTTCCGAGGTGATTTTGGCAATCTGTCAGCCTTAGAAAAACCAATCTATGATA



GCAAAGACGGTAGCAGCTACTATCATGAAGCAAATAGTGATGCCAAAGACCAATTAGATGGTGATGGA  
 TTACAGTTTTTACGCTCTGAAAAATAACTTTACAGCACTTACCACAGAGTCTAACCCATGGACGATTAT  
 TAAAGCTGTCAAAGAAGGGGTTGAAAACATAGAGGATATCGAATCTTCAGAGATCACAGAAACCATTT  
 TTGCAGGTACTTTTGCAAAACAAGACGATGATAGCCACTACTATATCCACCGTCACGCTAATGGCAAA  
 5 CCATATGCTGCGATCTCTCAAATGGGGACGGTAACAGAGATTATGTCCAATTCGAAGGTACTTTCTT  
 GCGTAATGCTAAAAACCTTGTGGCTGAAGTCTTGGACAAAGAAGGAAATGTTGTTTGGACAAGTGAGG  
 TAACCGAGCAAGTTGTTAAAAACTACAACAATGACTTGGCAAGCACACTTGGTTCAACCCGTTTTTGAA  
 AAAACGCGTTGGGACGGTAAAGATAAAGACGGCAAAGTTGTTGCTAACGGAACCTACACCTATCGTGT  
 TCGCTACACGCCGATTAGCTCAGGTGCAAAAAGAACAACACACTGATTTTGTATGTGATTGTAGACAATA  
 10 CGACACCTGAAGTCGCAACATCGGCAACATTCTCAACAGAAGATAGTCGTTTGGACACTTGCATCTAAA  
 CCAAAAACCAGCCAACCGGTTTACCGTGAGCGTATTGCTTACACTTATATGGATGAGGATCTGCCAAC  
 AACAGAGTATATTTCTCAAATGAAGATGGTACCTTTACTCTTCCTGAAGAGGCTGAAACAATGGAAG  
 GCGCTACTGTTCCATTGAAAATGTCAGACTTTACTTATGTTGTTGAAGATATGGCTGGTAACATCACT  
 TATACACCAGTGACTAAGCTATTGGAGGGCCACTCTAATAAGCCAGAACAAGACGGTTCAGATCAAGC  
 15 ACCAGACAAGAAACCAGAAGCTAAACCAGAACAAGACGGTTCAGGTCAAACACCAGATAAAAAAAG  
 AACTAAACCAGAAAAAGATAGTTTCAGGTCAAACACCAGGTAAACTCCTCAAAAAGGTCAATCTTCT  
 CGTACTCTAGAGAAACGATCTTCTAAGCGTGCTTTAGCTACAAAAGCATCAACAAGAGATCAGTTACC  
 AACGACTAATGACAAGGATACAAATCGTTTACATCTCCTTAAGTTAGTTATGACCACTTTCTTCTTGG  
 GA

# **SEQ ID NO. 27**

MRKKQKLPFDKLAIALISTSIILLNAQSDIKANTVTEFTPATEQAVEPPQPIAVSEESRSSKETKTSQT  
 PSDVGETVADDANDLAPQAPAKTADTPATSKATIRDLNDPSHVKTLOEKAGKGAGTVVAVIDAGFDKN  
 HEAWRLTDKTKARYQSKENLEKAKKEHGITYGEWVNDKVAYYHDYSKDGKNAVDQEHGTHVSGILSGN  
 25 APSEMKEPYRLEGAMPEAQLLLMRVEIVNGLADYARNYAQAIRDAVNLGAKVINMSFGNAALAYANLP  
 DETKKAFFDYAKSKGVSIIVTSAGNDSSFSGGKPRPLADHPDYGVVGTAAADSTLTVASYSYSPDKQLTET  
 ATVKTDHDDHDKEMPVISTNRFEFNPAYDYAYANRGTKEDEDFKDVEGKIALIERGDI DFKDKIANAKKA  
 GAVGVL IYDNQDKGFPIELPNVDQMPAAFISRRDGLLLKDNPPKTIITFNATPKVLPASGTKLSRFSS  
 WGLTADGNIKPDIAAPGQDILSSVANNKYAKLSGTSMSAPLVAGIMGLLQKQYETQYPDMPSERLDL  
 30 AKKVLMS SATALYDEDEKAYFSPRQQGAGAVDAKKASAATMYVTDKDNNTSSKVHLNNVSDKFEVTVTV  
 HNKSDKPQELYQVTVQTDKVDGKH FALAPKALYETSWQKITIPANSSKQVTVPIDASRFSKDLLAQM  
 KNGYFLEGGFVRFKQDPTKEELMSIPIYGFRGDFGNLSALEKPIYDSKDGSSYYHEANSDAKDQLDGDG  
 LQFYALKNNFTALTTESNPWTIIKAVKEGVENIEDIESSEITETIFAGTFAKQDDDSHYIHRHANGK  
 PYAAISPNGDGNRDYVQFQGTFLRNAKNLVAEVL DKEGNVVTSEVTEQVVKNNNDLASTLGSTRFE  
 35 KTRWDGKDKDGKVVANGTYTYRVRYTPISSGAKEQHTDFDVIVDNTTPEVATSATFSTEDSRLTLASK  
 PKTSQPVYRERIAAYTMDLPTTEYISPNEGTFLLPEEAETMEGATVPLKMSDFTYVVEDMAGNIT  
 YTPVTKLLEGHSSNKPEQDGS DQAPDKKPEAKPEQDGSQTPDKKKETKPEKDSSGQTPGKTPQKGQSS  
 RTLEKRSSKRALATKASTRDQLPTTNDKDTNRLHLLKLVMTTFFLG

40 GBS 276 contains an N-terminal leader or signal sequence region which is indicated by the underlined sequence at the beginning of SEQ ID NO: 27 above. In one embodiment, one or more amino acids from the leader or signal sequence region of GBS 276 are removed. An example of such a GBS 276 fragment is set forth below as SEQ ID NO: 28.

**SEQ ID NO: 28**

QSDIKANTVTEDTPATEQAVEPPQPIAVSEESRSSKETKTSQTPSDVGETVADDANDLAPQAPAKTAD  
 TPATSKATIRDLNDPSHVKTLLQEKAGKGAGTVVVAVIDAGFDKNHEAWRLTDKTKARYQSKENLEKAKK  
 5 EHGITYGEWVNDKVAYYYHDYSKDGKNAVDQEHGTHVSGILSGNAPSEMKEPYRLEGAMPEAQLLLLMRV  
 EIVNGLADYARNYAQAIRDAVNLGAKVINMSFGNAALAYANLPDETKKAFDYAKSKGVSIVTSAGNDS  
 SFGGKPRLLPLADHPDYGVVGTAAADSTLTVASYSPPDKQLTETATVKTDDHQDKEMPVISTNRFEPNK  
 AYDYAYANRGTKEDDFKDVEGKIALIERGDIIDFKDKIANAKKAGAVGVLIYDNQDKGFPIELPNVDQM  
 10 PAAFI SRRDGLLLKDNPPKTITFNATPKVLPTASGTKLSRFSSWGLTADGNIKPDI AAPGQDILSSVA  
 NNKYAKLSGTSMSAPLVAGIMGLLQKQYETQYPDMTPSERLDLAKKVLMS SATALYDEDEKAYFS PRQ  
 QGAGAVDAKKASAATMYVTDKNTSSKVHLNNVSDKFEVTVTVHNKSDKPQELYQVTVQTDKVDGKH  
 FALAPKALYETSWQKITIPANSSKQVTVPIDASRFSKDLAQMKNKYFLEGFVRFKQDPTKEELMSIP  
 YIGFRGDFGNLSALEKPIYDSKDGSSYYHEANSDAKDQLDGDGLQFYALKNNFTALTTESNPWTIIKA  
 15 VKEGVENIEDIESSEITETIFAGTFAKQDDDSHYIHRHANGKPYAAISPNGDGNRDYVQFQGTFLRN  
 AKNLVAEVL DKEGNVVTSEVTEQVVKNNNDLASTLGSTRFEKTRWDGKDKDGKVVANGTYTYRVRY  
 TPISSGAKEQHTDFDVI DVNTTPEVATSATFSTEDSRLTLASKPKTSQP VYRERIA YTYMDEDLPTTE  
 YISPNEDGTFTLPEEAETMEGATVPLKMSDFTYVVEDMAGNITYTPVTKLLEGHSNKPEQDGS DQAPD  
 20 KKPEAKPEQDGSQTPDKKKETKPEKDSSGQTPGKTPQKGQSSRTLEKRSSKRALATKASTRDQLPTT  
 NDKDTNRLHLLKLVMTTFFLG

GBS 276 contains a C-terminal transmembrane and/or cytoplasmic region which is indicated  
 by the underlined sequence near the end of SEQ ID NO: 27 above. In one embodiment, one or more  
 amino acids from the transmembrane or cytoplasmic regions of GBS 276 are removed. An example  
 of such a GBS 276 fragment is set forth below as SEQ ID NO: 29.

**SEQ ID NO: 29**

MRKKQKL PFDKLAIALISTSI LLNAQSDIKANTVTEDTPATEQAVEPPQPIAVSEESRSSKETKTSQTP  
 PSDVGETVADDANDLAPQAPAKTADTPATSKATIRDLNDPSHVKTLLQEKAGKGAGTVVVAVIDAGFDKN  
 HEAWRLTDKTKARYQSKENLEKAKKEHGITYGEWVNDKVAYYYHDYSKDGKNAVDQEHGTHVSGILSGN  
 30 APSEMKEPYRLEGAMPEAQLLLLMRVEIVNGLADYARNYAQAIRDAVN LGAKVINMSFGNAALAYANLP  
 DETKKA FDYAKSKGVSIVTSAGNDS SFGGKPRLLPLADHPDYGVVGTAAADSTLTVASYSPPDKQLTET  
 ATVKTDDHQDKEMPVISTNRFEPNKAYDYAYANRGTKEDDFKDVEGKIALIERGDIIDFKDKIANAKKA  
 GAVGVLIYDNQDKGFPIELPNVDQM PAAFI SRRDGLLLKDNPPKTITFNATPKVLPTASGTKLSRFSS  
 35 WGLTADGNIKPDI AAPGQDILSSVANNKYAKLSGTSMSAPLVAGIMGLLQKQYETQYPDMTPSERLDL  
 AKKVLMS SATALYDEDEKAYFS PRQQGAGAVDAKKASAATMYVTDKNTSSKVHLNNVSDKFEVTVTV  
 HNKS DKPQELYQVTVQTDKVDGKH FALAPKALYETSWQKITIPANSSKQVTVPIDASRFSKDLAQM  
 KNGYFLEGFVRFKQDPTKEELMSIPYIGFRGDFGNLSALEKPIYDSKDGSSYYHEANSDAKDQLDGDG  
 LQFYALKNNFTALTTESNPWTIIKAVKEGVENIEDIESSEITETIFAGTFAKQDDDSHYIHRHANGK  
 PYAAISPNGDGNRDYVQFQGTFLRNAKNLVAEVL DKEGNVVTSEVTEQVVKNNNDLASTLGSTRFE  
 40 KTRWDGKDKDGKVVANGTYTYRVRYTPISSGAKEQHTDFDVI DVNTTPEVATSATFSTEDSRLTLASK  
 PKTSQP VYRERIA YTYMDEDLPTTEYISPNEDGTFTLPEEAETMEGATVPLKMSDFTYVVEDMAGNIT  
 YTPVTKLLEGHSNKPEQDGS DQAPDKKPEAKPEQDGSQTPDKKKETKPEKDSSGQTPGKTPQKGQSS  
 RTLEKRSSKRALATK

In one embodiment, one or more amino acids from the leader or signal sequence region and  
 one or more amino acids from the transmembrane or cytoplasmic regions of GBS 276 are removed.  
 An example of such a GBS 276 fragment is set forth below as SEQ ID NO: 30.

**SEQ ID NO: 30**

QSDIKANTVTEDTPATEQAVEPPQPIAVSEESRSSKETKTSQTPSDVGETVADDANDLAPQAPAKTAD  
 TPATS KATIRDLNDPSHVKTLOEKAGKGAGTVVAVIDAGFDKNHEAWRLTDKTKARYQSKENLEKAKK  
 EHGITYGEWVNDKVAYYHDYSKDGKNAVDQEHGTHVSGILSGNAPSEMKEPYRLEGAMPEAQLLLMRV  
 EIVNGLADYARNYAQAIRDAVNLGAKVINMSFGNAALAYANLPDETKKAFDYAKSKGVSIVTSAGNDS  
 5 SFGGKPRPLADHPDYGVVGTAAADSTLTVASYS PDKQLTETATVKTDHDDHDKEMPVISTNRFEPNK  
 AYDYAYANRGTKEDDFKDVEGKIALIERGDIIDFKDKIANAKKAGAVGVLIYDNQDKGFPIELPNVDQM  
 PAAFI SRRDGLLLKDNPPKTITFNATPKVLPTASGTKLSRFSSWGLTADGNIKPDIAAPGQDILSSVA  
 NNKYAKLSGTSMSAPLVAGIMGLLQKQYETQYPDMTPSERLDLAKKVLMS SATALYDEDEKAYFS PRQ  
 10 QGAGAVDAKKASAATMYVTDKDNSTSSKVHLNNVSDKFEVTVTVHNKSDKPQELYQVTVQTDKVDGKH  
 FALAPKALYETSWQKITIPANSSKQVTPIDASRFSKDLLAQMKNGYFLEGFVRFKQDPTKEELMSIP  
 YIGFRGDFGNLSALEKPIYDSKDGSSYYHEANSDAKDQLDGLQFYALKNNFTALTTESNPWTIIKA  
 VKEGVENIEDIESSEITETIFAGTFAKQDDDSHYIHRHANGKPYAAISPNGDGNRDYVQFQGTFLRN  
 AKNLVAEVL DKEGNVVTSEVTEQVVKYNNNDLASTLGSTRFEKTRWDGKDKDGKVVANGTYTYRVRY  
 15 TPISSGAKEQHTDFDVIVDNTTPEVATSATFSTEDSRLTLASKPKTSQPVYRERIAYTYMDEDLPTE  
 YISPNEGDGTFLLPEEAETMEGATVPLKMSDFTYVVEDMAGNITYTPVTKLLEHGSNKPEQDGSQAPD  
 KKPEAKPEQDGSQGQTPDKKKETKPEKDSSGQTPGKTPQKGQSSRTLEKRSSKRALATK

Further description of GBS 276 can be found in the following references: Qi Chen et al.,  
 “Immunization with C5a Peptidase or Peptidase-Type III Polysaccharide conjugate Vaccines  
 20 Enhances Clearance of Group B Streptococci from Lungs of Infected Mice”, *Infection and Immunity*  
 (2002) 70 (11):6409 – 6415; Beckmann et al., “Identification of Novel Adhesions from Group B  
 Streptococci by Use of Phage Display Reveals that C5a Peptidase Mediates Fibronectin Binding”  
*Infection and Immunity* (2002) 70(6):2869 – 2876; Cheng et al., “The Group B Streptococcal C5a  
 Peptidase Is Both a Specific Protease and an Invasin” *Infection and Immunity* (2002) 70(5) 2408 –  
 25 2413; and Cheng et al., “Antibody against Surface-Bound C5a Peptidase Is Opsonic and Initiates  
 Macrophage Killing of Group B Streptococci” *Infection and Immunity* (2001) 69(4):2302 – 2308.

### GBS 305

GBS 305 refers to a UDP-N-acetylmuramoylalanine--D-glutamate ligase, also referred to  
 30 as Mur D. Nucleotide and amino acid sequences of GBS 305 sequenced from serotype V isolated  
 strain 2603 V/R are set forth in Ref. 2 as SEQ ID 207 and SEQ ID 208. These sequences are set forth  
 below as SEQ ID NOS 31 and 32:

### **SEQ ID NO. 31**

35 ATGGGACGAGTAATGAAAACAATAACAACATTTGAAAATAAAAAAGTTTTAGTCCTTGGTTTAGCACG  
 ATCTGGAGAAGCTGCTGCACGTTTGTAGCTAAGTTAGGAGCAATAGTGACAGTTAATGATGGCAAAC  
 CATTTGATGAAAATCCAACAGCACAGTCTTTGTTGGAAGAGGGTATTAAAGTGGTTTGTGGTAGTCAT  
 CCTTTAGAATTGTTAGATGAGGATTTTTGTTACATGATTAAAAATCCAGGAATACCTTATAACAATCC  
 TATGGTCAAAAAAGCATTAGAAAAACAAATCCCTGTTTTGACTGAAGTGGAATTAGCATACTTAGTTT  
 40 CAGAACTCTCAGCTAATAGGTATTACAGGCTCTAACGGGAAAACGACAACGACAACGATGATTGCAGAA  
 GTCTTAAATGCTGGAGGTCAGAGAGGTTTGTAGCTGGGAATATCGGCTTTCCTGCTAGTGAAGTTGT  
 TCAGGCTGCGAATGATAAAGATACTCTAGTTATGGAATTATCAAGTTTTCAGCTAATGGGAGTTAAGG  
 AATTTGCTCCTCATATTGCAGTAATTACTAATTTAATGCCAACTCATTTAGATTATCATGGGTCTTTT  
 45 GAAGATTATGTTGCTGCAAAATGGAATATCCAAAATCAAATGCTTCATCTGATTTTTTGGTACTTAA  
 TTTTAATCAAGGTATTTCTAAAGAGTTAGCTAAAATACTAAAGCAACAATCGTTTCTTCTCTACTA  
 CGGAAAAAGTTGATGGTGCTTACGTACAAGACAAGCAACTTTTCTATAAAGGGGAGAATATTATGTCA

GTAGATGACATTGGTGTCCCAGGAAGCCATAACGTAGAGAATGCTCTAGCAACTATTGCGGTTGCTAA  
 ACTGGCTGGTATCAGTAATCAAGTTATTAGAGAACTTTAAGCAATTTTGGAGGTGTTAAACACCGCT  
 TGCAATCACTCGGTAAGGTTTCATGGTATTAGTTTCTATAACGACAGCAAGTCAACTAATATATTGGCA  
 ACTCAAAAAGCATTATCTGGCTTTGATAATACTAAAGTTATCCTAATTGCAGGAGGTCTTGATCGCGG  
 5 TAATGAGTTT GATGAATTGATACCAGATATCACTGGACTTAAACATATGGTTGTTT TAGGGGAATCGG  
 CATCTCGAGTAAAACGTGCTGCACAAAAGCAGGAGTAACTTATAGCGATGCTTTAGATGTTAGAGAT  
 GCGGTACATAAAGCTTATGAGGTGGCACAACAGGGCGATGTTATCTTGCTAAGTCCTGCAAATGCATC  
 ATGGGACATGTATAAGAATTTCAAGTCCGTGGTGATGAATTCATTGATACTTTCGAAAGTCTTAGAG  
 GAGAG

**SEQ ID NO. 32**

MGRVMKTITTT FENKKVLVLGLARSGEAAARLLAKLGAIIVTVNDGKPF DENPTAQSLLEEGIKVVC GSH  
 PLELLDEDFCYMIKNPGIPYNNPMVKKALEKQIPVLTEVELAYLVSESQ LIGITGSNGKTTTTT MIAE  
 VLNAGGQRGLLAGNIGFPASEVVQAANDKDTLVMELSSFQLMGVKEFRPHIAVITNLMPTHLDYHGSF  
 15 EDYVAAKWNIQNQMS SDFLVLNFNQGISKE LAKTTKATIVPFSTTEKVDGAYVQDKQLFYKGENIMS  
 VDDIGVPGSHNVENALATI AVAKLAGISNQVIRETLSNFGGVK HRLQSLGKVHGISFYND SKSTNILA  
 TQKALSGFDNTKVIL IAGGLDRGNEFDELI PDITGLKHMVVLGESASRVKRAAQKAGVTYS DALDVRD  
AVHKAYEVAQQGDVILLSPANASWDMYKNFEVRGDEFIDTFESLRGE

20 GBS 305 contains an N-terminal leader or signal sequence region which is indicated by the  
 underlined sequence at the beginning of SEQ ID NO: 32 above. In one embodiment, one or more  
 amino acids from the leader or signal sequence region are removed from GBS 305. An example of  
 such a GBS 305 fragment is set forth below as SEQ ID NO: 33.

**SEQ ID NO: 33**

ITTFENKKVLVLGLARSGEAAARLLAKLGAIIVTVNDGKPF DENPTAQSLLEEGIKVVC GSHPLELLDE  
 DFCYMIKNPGIPYNNPMVKKALEKQIPVLTEVELAYLVSESQ LIGITGSNGKTTTTT MIAEVLNAGGQ  
 RGLLAGNIGFPASEVVQAANDKDTLVMELSSFQLMGVKEFRPHIAVITNLMPTHLDYHGSFEDYVAAK  
 25 WNIQNQMS SDFLVLNFNQGISKE LAKTTKATIVPFSTTEKVDGAYVQDKQLFYKGENIMSVDDIGVP  
 30 GSHNVENALATI AVAKLAGISNQVIRETLSNFGGVK HRLQSLGKVHGISFYND SKSTNILATQKALSG  
 FDNTKVIL IAGGLDRGNEFDELI PDITGLKHMVVLGESASRVKRAAQKAGVTYS DALDVRDAVHKAYE  
 VAQQGDVILLSPANASWDMYKNFEVRGDEFIDTFESLRGE

35 GBS 305 contains a C-terminal transmembrane or cytoplasmic region indicated by the  
 underlined sequence near the end of SEQ ID NO: 32 above. In one embodiment, one or more amino  
 acids from the transmembrane or cytoplasmic regions are removed from GBS 305. An example of  
 such a GBS 305 fragment is set forth below as SEQ ID NO: 34.

**SEQ ID NO: 34**

40 MGRVMKTITTT FENKKVLVLGLARSGEAAARLLAKLGAIIVTVNDGKPF DENPTAQSLLEEGIKVVC GSH  
 PLELLDEDFCYMIKNPGIPYNNPMVKKALEKQIPVLTEVELAYLVSESQ LIGITGSNGKTTTTT MIAE  
 VLNAGGQRGLLAGNIGFPASEVVQAANDKDTLVMELSSFQLMGVKEFRPHIAVITNLMPTHLDYHGSF  
 EDYVAAKWNIQNQMS SDFLVLNFNQGISKE LAKTTKATIVPFSTTEKVDGAYVQDKQLFYKGENIMS  
 45 VDDIGVPGSHNVENALATI AVAKLAGISNQVIRETLSNFGGVK HRLQSLGKVHGISFYND SK

In one embodiment one or more amino acids from the leader or signal sequence region and one or more amino acids from the transmembrane or cytoplasmic regions are removed from GBS 305. An example of such a GBS 305 fragment is set forth below as SEQ ID NO: 35.

#### 5 **SEQ ID NO: 35**

ITTFENKKVLVLGLARSGEAAARLLAKLGAIIVTVNDGKPFDENPTAQSLLLEEGIKVVCGSHPLELLDE  
DFCYMIKNPGIPYNNPMVKKALEKQIPVLTEVELAYLVSESQILIGITGSNGKTTTTMTIAEVLNAGGQ  
RGLLAGNIGFPASEVVQAANDKDTLVMELSSFQLMGVKEFRPHIAVITNLMPTHLDYHGSFEDYVAAK  
10 WNIQNQMSSSDFLVLNFNQGISKEKAKTTKATIVPFSTTEKVDGAYVQDKQLFYKGENIMSVDDIGVP  
GSHNVENALATIIVAKLAGISNQVIRETLSNFGGVKHLQSLGKVHGISFYNDK

#### GBS 322

GBS 322 refers to a surface immunogenic protein, also referred to as "sip". Nucleotide and amino acid sequences of GBS 322 sequenced from serotype V isolated strain 2603 V/R are set forth in  
15 Ref. 2 as SEQ ID 8539 and SEQ ID 8540. These sequences are set forth below as SEQ ID NOS 36  
and 37:

#### **SEQ ID NO. 36**

ATGAATAAAAAGGTACTATTGACATCGACAATGGCAGCTTCGCTATTATCAGTCGCAAGTGTTCAAGC  
20 ACAAGAAACAGATACGACGTGGACAGCACGTACTGTTTCAGAGGTAAAGGCTGATTTGGTAAAGCAAG  
ACAAATAATCATCATATACTGTGAAATATGGTGATACACTAAGCGTTATTTTCAAGCAATGTCAATT  
GATATGAATGTCTTAGCAAAAATAAATAACATTGCAGATATCAATCTTATTTATCCTGAGACAACACT  
GACAGTAACTTACGATCAGAAGAGTCATACTGCCACTTCAATGAAAATAGAAACACCAGCAACAAATG  
CTGCTGGTCAAACACAGCTACTGTGGATTTGAAAACCAATCAAGTTTCTGTTGCAGACCAAAAAGTT  
25 TCTCTCAATACAATTTTCGGAAGGTATGACACCAGAAGCAGCAACAACGATTGTTTCGCCAATGAAGAC  
ATATTTCTTCTGCGCCAGCTTTGAAATCAAAGAAGTATTAGCACAAGAGCAAGCTGTTAGTCAAGCAG  
CAGCTAATGAACAGGTATCACCAGCTCCTGTGAAGTCGATTACTTTCAGAAGTTCCAGCAGCTAAAGAG  
GAAGTTAAACCAACTCAGACGTCAGTCAGTCAGTCAACAACAGTATCACCAGCTTCTGTTGCCGCTGA  
AACACCAGCTCCAGTAGCTAAAGTAGCACCAGGTAAGAAGTGTAGCAGCCCCTAGAGTGGCAAGTGTTA  
30 AAGTAGTCACTCCTAAAGTAGAACTGGTGCATCACCAGAGCATGTATCAGCTCCAGCAGTTTCTGTG  
ACTACGACTTCACCAGCTACAGACAGTAAGTTACAAGCGACTGAAGTTAAGAGCGTTCCGGTAGCACA  
AAAAGCTCCAACAGCAACACCGGTAGCACAACCAGCTTCAACAACAAATGCAGTAGCTGCACATCCTG  
AAAATGCAGGGCTCCAACCTCATGTTGCAGCTTATAAAGAAAAAGTAGCGTCAACTTATGGAGTTAAT  
GAATTCAGTACATAACCGTGCAGGAGATCCAGGTGATCATGGTAAAGGTTTAGCAGTTGACTTTATTGT  
35 AGGTACTAATCAAGCACTTGGTAATAAAGTTGCACAGTACTCTACACAAAATATGGCAGCAATAACA  
TTTCATATGTTATCTGGCAACAAAAGTTTTACTCAAATACAAACAGTATTTATGGACCTGCTAATACT  
TGGAATGCAATGCCAGATCGTGGTGGCGTTACTGCCAACCACTATGACCACGTTACGTATCATTTAA  
CAAAATAATATAAAAAAGGAAGCTATTTGGCTTCTTTTTTATATGCCTTGAATAGACTTTCAAGGTTCT  
TATAATAATTTTTATTA

40

#### **SEQ ID NO. 37**

MNKKVLLTSTMAASLLSVASVQAQETDTTWTARTVSEVKADLVKQDNKSSYTVKYGDTLSVISEAMSI  
DMNVLAKINNIADINLIYPETTLTVTYDQKSHTATSMKIETPATNAAGQTTATVDLKTNQVSVADQKV  
SLNTISEGMTPEAATTIVSPMKTYSSAPALKSKEVLAQEQAVSQAAANEQVSPAPVKSITSEVPAAKE  
45 EVKPTQTSVSQSTTVSPASVAAETPAPVAKVAPVRTVAAPRVASVKVVT PKVETGASPEHVSAPVPV  
TTTSPATDSKLQATEVKSVPVAQKAPTATPVAQPASTTNAVAHPENAGLQPHVAAYKEKVASTYGVN  
EFSTYRAGDPGDHKGGLAVDFIVGTNQALGNKVAQYSTQNMAANNISYVIWQQKFYSNTNSIYGPANT  
WNAMPDRGGVTANHYDHVHVSFNK

GBS 322 contains an N-terminal leader or signal sequence region which is indicated by the underlined sequence near the beginning of SEQ ID NO: 37. In one embodiment, one or more amino acids from the leader or signal sequence region of GBS 322 are removed. An example of such a GBS 322 fragment is set forth below as SEQ ID NO: 38.

**SEQ ID NO: 38**

DLVKQDNKSSYTVKYGDTLSVISEAMSIDMNVLAKINNIADINLIYPETTLTVTYDQKSHTATSMKIE  
TPATNAAGQTTATVDLKTNQVSVADQKVS LNTISEGMTPEAATTIVSPMKTYSSAPALKSKEVLAQEQ  
AVSQAAANEQVSPAPVKSITSEVPAAKEEVKPTQTSVVSQSTTVSPASVAAETPAPVAKVAPVRTVAAP  
RVASVKVVT PKVETGASPEHVSAPAVPVTTTSPATDSKLQATEVKSV PVAQKAPTATPVAQPASTTNA  
VAAHPENAGLQPHVAAAYKEKVASTYGVNEFSTYRAGDPGDH GKGLAVDFIVGTNQALGNKVAQYSTQN  
MAANNISYVIWQQKFYSNTNSIYGPANTWNPDRGGVTANHYDHVHVSFNK

**GBS 330**

GBS 330 refers to a pyruvate kinase, also referred to as "pyk". Nucleotide and amino acid sequences of GBS 330 sequenced from serotype V isolated strain 2603 V/R are set forth in Ref. 2 as SEQ ID 8791 and SEQ ID 8792. These sequences are set forth below as SEQ ID NOS 39 and 40:

**SEQ ID NO. 39**

ATGAATAAACGCGTAAAAATCGTTGCAACACTTGGTCCTGCGGTTGAATTCGGTGGTGAAGAAGTT  
TGGTGAGTCTGGATACTGGGGTGAAAGCCTTGACGTAGAAGCTTCAGCAGAAAAAATTGCTCAATTGA  
TTAAAGAAGGTGCTAACGTTTTCCGTTTTCAACTTCTCACATGGAGATCATGCTGAGCAAGGAGCTCGT  
ATGGCTACTGTTTCGTAAAGCAGAAGAGATTGCAGGACAAAAAGTTGGCTTCCTCCTTGATACTAAAGG  
ACCTGAAATTCGTACAGAACTTTTTGAAGATGGTGCAGATTTCCATTCATATACAACAGGTACAAAAT  
TACGTGTTGCTACTAAGCAAGGTATCAAATCAACTCCAGAAGTGATTGCATTGAATGTTGCTGGTGGGA  
CTTGACATCTTTGATGACGTTGAAGTTGGTAAGCAAATCCTTGTTGATGATGGTAAACTAGGTCTTAC  
TGTGTTTTGCAAAAGATAAAGACACTCGTGAATTTGAAGTAGTTGTTGAGAATGATGGCCTTATTGGTA  
AACAAAAAGGTGTAAACATCCCTTATACTAAAATTCCTTTCCAGCACTTGCAGAACGCGATAATGCT  
GATATCCGTTTTTGGACTTGAGCAAGGACTTAACTTTATTGCTATCTCATTGTACGTACTGCTAAAGA  
TGTTAATGAAGTTTCGTGCTATTTGTGAAGAACTGGSMATGGACACGTTAAGTTGTTTGCTAAAATTG  
AAAATCAACAAGGTATCGATAATATTGATGAGATTATCGAAGCAGCAGATGGTATTATGATTGCTCGT  
GGTGATATGGGTATCGAAGTTCCATTTGAAATGGTTCAGTTTACCAAAAAATGATCATTACTAAAGT  
TAATGCAGCTGGTAAAGCAGTTATTACAGCAACAAATATGCTTGAAACAATGACTGATAAACCACGTG  
CGACTCGTTCAGAAGTATCTGATGTCTTCAATGCTGTTATTGATGGTACTGATGCTACAATGCTTTCA  
GGTGAGTCAGCTAATGGTAAATACCCAGTTGAGTCAGTTCTGCATTCACGATTCCACGTAATAACAAAATGATG  
TCAACATTACTCAATGAGTATGGTCGCTTAGACTCATCTGCATTCACGATTCCACGTAATAACAAAATGATG  
TTATTGCATCTGCGGTTAAAGATGCAACACACTCAATGGATATCAAATGTTGTAACAATTACTGAA  
ACAGGTAATACAGCTCGTGCCATTTCTAAATTCCGTCCAGATGCAGACATTTTGGCTGTTACATTTGA  
TGAAAAAGTACAACGTTTATTGATGATTAATGTTGTTATCCCTGTCCTTGACAGACAAACCAGCAT  
CTACAGATGATATGTTTGAGGTTGCAGAACGTGTAGCACTTGAAGCAGGATTTGTTGAATCAGGCGAT  
AATATCGTTATCGTTGCAGGTGTTCTGTAGGTACAGGTGGAACATAACACAATGCGTGTTTCGTACTGT  
TAAA

**SEQ ID NO. 40**

MNKRVKIVATLGPAVEFRGGKKFGESGYWGESLDVEASAEKIAQLIKEGANVFRFNFSHGDHAEQGAR  
 MATVRKAEEIAGQKVGFLLDTKGPEIRTELFEDGADFHSYTTGTKLRVATKQGIKSTPEVIALNVAGG  
 5 LDI FDDVEVGKQILVDDGKLGLTVFAKDKDTREFEVVVENDGLIGKQKGVNIPYTKIPFPALAERDNA  
 DIRFGLEQGLNFIAISFVRTAKDVNEVRAICEETGXGHVKLFAKIENQQGIDNIDEIIEAADGIMIAR  
 GDMGIEVPFEMVPVYQKMIITKVNAAGKAVITATNMLETMTDKPRATRSEVSDVFNVIDGTDATMLS  
 GESANGKYPVESVRTMATIDKNAQTLLENYGRLDSSAFPRNNKTDVIASAVKDATHSMDIKLVVTITE  
 10 TGNTARAISKFRPDADILAVTFDEKVVQRSLMINWGVIPVLADKPASTDDMFEVAERVALEAGFVESGD  
 NIVIVAGVPVGTGGTNTMRVRTVK

**GBS 338**

GBS 338 refers to a Sat D protein. Nucleotide and amino acid sequences of GBS 338  
 sequenced from serotype V isolated strain 2603 V/R are set forth in Ref. 2 as SEQ ID 8637 and SEQ  
 15 ID 8638. These sequences are set forth below as SEQ ID NOS 41 and 42:

**SEQ ID NO. 41**

TTGTCTGCTATAATAGACAAAAAGGTGGTGATATTTATGTATTTAGCATTAATCGGTGATATCATTA  
 TTCAAAACAGATACTTGAACGTGAAACTTTCCAACAGTCTTTTCAGCAACTAATGACCGAACTATCTG  
 20 ATGTATATGGTGAAGAGCTGATTTCTCCATTCACATTTACAGCTGGTGATGAATTTCAAGCTTTATTG  
 AAACCATCAAAAAAGGTATTTCAAATTATTGACCATATTCAACTAGCTCTAAAACCTGTTAATGTAAG  
 GTTCGGCCTCGGTACAGGAAACATTATAACATCCATCAATTCAAATGAAAGTATCGGTGCTGATGGTC  
 CTGCCTACTGGCATGCTCGCTCAGCTATTAATCATATACATGATAAAAAATGATTATGGAACAGTTCAA  
 GTAGCTATTTGCCTTGATGATGAAGACCAAAACCTTGAATTAACACTAAATAGTCTCATTTTCAGCTGG  
 25 TGATTTTATCAAGTCAAATGGACTACAAACCATTTTCAAATGCTTGAGCACTTAATACTTCAAGATA  
 ATTATCAAGAACAATTTCAACATCAAAAGTTAGCCCAACTGGAAAATATTGAACCTAGTGCGCTGACT  
 AAACGCCTTAAAGCAAGCGGTCTGAAGATTTACTTAAGAACGAGAACACAGGCAGCCGATCTATTAGT  
 TAAAAGTTGCACTCAAACATAAGGGGGAAGCTATGATTTT

**SEQ ID NO. 42**

MSAIIDKKVVIFMYLALIGDIINSKQILERETFQQS FQQLMTELSDVYGEELISPFTITAGDEFQALL  
 KPSKKVFQIIDHIQLALKPVNVRFGLTGNIITSINSNESIGADGPAYWHARSAINHIHDKNNDYGT  
 35 VQVAICLDDEDQNL ELTLNSLISAGDFIKSKWTTNHFQMLEHLILQDNYQE QFQHQKLAQLENIEPSALT  
 KRLKASGLKIYLRTRTQAADLLVKSCTQTKGGSYDF

GBS 338 may contain an N-terminal leader or signal sequence region which is indicated by  
 the underlined sequence at the beginning of SEQ ID NO: 42 above. In one embodiment, one or more  
 amino acids from the leader or signal sequence region are removed from GBS 338. An example of  
 such a GBS 338 fragment is set forth below as SEQ ID NO: 43.

**SEQ ID NO: 43**

MYLALIGDIINSKQILERETFQQS FQQLMTELSDVYGEELISPFTITAGDEFQALLKPSKKVFQIIDH  
 IQLALKPVNVRFGLTGNIITSINSNESIGADGPAYWHARSAINHIHDKNNDYGT VQVAICLDDEDQNL  
 45 ELTLNSLISAGDFIKSKWTTNHFQMLEHLILQDNYQE QFQHQKLAQLENIEPSALT KRLKASGLKIYLR  
 TRTQAADLLVKSCTQTKGGSYDF

GBS 361

GBS 361 refers to a *cylI* protein. Nucleotide and amino acid sequences of GBS 361 sequenced from serotype V isolated strain 2603 V/R are set forth in Ref. 2 as SEQ ID 8769 and SEQ ID 8770. These sequences are set forth below as SEQ ID NOS 44 and 45:

**SEQ ID NO. 44**

ATGAGCGTATATGTTAGTGGAATAGGAATTATTTCTTCTTTGGGAAAGAATTATAGCGAGCATAAACA  
GCATCTCTTCGACTTAAAAGAAGGAATTTCTAACATTTATATAAAAAATCACGACTCTATTTTAGAAT  
CTTATACAGGAAGCATAACTAGTGACCCAGAGGTTCTGAGCAATACAAAGATGAGACACGTAATTTT  
10 AAATTTGCTTTTACCGCTTTTGAAGAGGCTCTTGCTTCTTCAGGTGTTAATTTAAAAGCTTATCATAA  
TATTGCTGTGTGTTTAGGGACCTCACTTGGGGGAAAGAGTGCTGGTCAAATGCCTTGTATCAATTTG  
AAGAAGGAGAGCGTCAAGTAGATGCTAGTTTATTAGAAAAAGCATCTGTTTACCATATTGCTGATGAA  
TTGATGGCTTATCATGATATTGTGGGAGCTTCGTATGTTATTTCAACCGCCTGTTCTGCAAGTAATAA  
TGCCGTAATATTAGGAACACAATTACTTCAAGATGGCGATTGTGATTTAGCTATTTGTGGTGGCTGTG  
15 ATGAGTTAAGTGATATTTCTTTAGCAGGCTTCACATCACTAGGAGCTATTAATACAGAAATGGCATGT  
CAGCCCTATTCTTCTGGAAGGAATCAATTTGGGTGAGGGCGCTGGTTTTGTGTTCTTGTCAAAGA  
TCAGTCCTTAGCTAAATATGGAATAATTATCGGTGGTCTTATTACTTCAGATGGTTATCATATAACAG  
CACCTAAGCCAACAGGTGAAGGGGCGGCACAGATTGCAAAGCAGCTAGTGACTCAAGCAGGTATTGAC  
TACAGTGAGATTGACTATATTAACGGTCACGGTACAGGTACTCAAGCTAATGATAAAATGGAAAAAA  
20 TATGTATGGTAAGTTTTTCCCGACAACGACATTGATCAGCAGTACCAAGGGGCAAACGGGTCACTACTC  
TAGGGGCTGCAGGTATTATCGAATTGATTAATTGTTTAGCGGCAATAGAGGAACAGACTGTACCAGCA  
ACTAAAAATGAGATTGGGATAGAAGGTTTTCCAGAAAAATTTTGTCTATCATCAAAAGAGAGAATACCC  
AATAAGAAATGCTTTTAAATTTTTCTGTTTGTGTTTGGTGAAATAATAGTGGTGTCTTATTGTCATCTT  
TAGATTACCTCTAGAAACATTACCTGCTAGAGAAAATCTTAAATGGCTATCTTATCATCTGTTGCT  
25 TCCATTTCTAAGAATGAATCACTTTCTATAACCTATGAAAAAGTTGCTAGTAATTTCAACGACTTTGA  
AGCATTACGCTTTTAAAGGGGCTAGACCACCCAAAACGTCAACCCAGCACAAATTTAGGAAAATGGATG  
ATTTTTCCAAAATGGTTGCCGTAACAACAGCTCAAGCACTAATAGAAAGCAATATTAATCTAAAAAAA  
CAAGATACTTCAAAAGTAGGAATTGTATTTACAACACTTTCTGGACCAGTTGAGGTGTTGAAGGTAT  
TGAAAAGCAAATCACAACAGAAGGATATGCACATGTTTCTGCTTCACGATTCCCGTTTACAGTAATGA  
30 ATGCAGCAGCTGGTATGCTTTCTATCATTTTTTAAATAACAGGTCCTTTATCTGTCAATTTTCGACAAAT  
AGTGGAGCGCTTGATGGTATACAATATGCCAAGGAAATGATGCGTAACGATAATCTAGACTATGTGAT  
TCTTGTTTCTGCTAATCAGTGGACAGACATGAGTTTTATGTGGTGGCAACAATTAACCTATGATAGTC  
AAATGTTTGTGCGTTCTGATTATTGTTTCAGCACAAGTCCTCTCTCGTCAAGCATTGGATAATTCTCCT  
ATAATATTAGGTAGTAAACAATTAAAATATAGCCATAAAACATTACAGATGTGATGACTATTTTTGA  
35 TGCTGCGCTTCAAAATTTATTATCAGACTTAGGACTAACCATAAAAGATATCAAAGGTTTCTGTTTGGG  
ATGAGCGGAAGAAGGCAGTTAGTTTCAGATTATGATTTCTTAGCGAACTTGTCTGAGTATTATAATATG  
CCAAACCTTGCTTCTGGTCAGTTTGGATTTTCATCTAATGGTGTGGTGAAGAACTGGACTATACTGT  
TAATGAAAGTATAGAAAAGGGCTATTATTTAGTCCTATCTTATTTCGATCTTCGGTGGTATCTCTTTTG  
CTATTATTGAAAAAAGG

**SEQ ID NO. 45**

MSVYVSGIGIISSLGKNYSEHKQHLFDLKEGISKHLYKNHDSILES YTGSI TSDPEVPEQYKDETRNF  
KFAFTAFEEALASSGVNLKAYHNIIVCLGTS LGGKSAGQNALYQFEEGERQVDASLLEKASVYHIAD E  
LMAYHDIVGASYVISTACSASNNAVILGTQLLQDGDCLAI CGGCDELSDISLAGFTSLGAINTEMAC  
45 QPYSSGKGINLGEGAGFVVLVKDQSLAKYGKIIGGLITSDGYHITAPKPTGEGAAQIAKQLVTQAGID  
YSEIDYINGHGTGTQANDKMEKNMYGKFFPTTTLISSTKGQTGHTLGAAGIIE LINCLAAIEEQTVPA  
TKNEIGIEGFENFVYHQREYPIRNALNFSFAFGGNSGVLLSSLDSPLETL PARENLMKMAILSSVA  
SISKNESLSITYEKVASNFNDFEALRFK GARPPKTVNPAQFRKMDDFSKMVAVTTA QALIESNINLKK  
QDTSKVGIVFTT LSGPVEVVEGIEKQITTEGYAHVSASRFPFTVMNAAAGMLSII FKITGPLSVISTN  
50 SGALDGIQYAKEMMRNDNL DYVILV SANQWTDMSFMWWQQLNYDSQMFVGS DYCSAQVLSRQALDNSP



I ILGSKQLKYSHKTFDVTMTIFDAALQNLLSDLGLTIKDIKGFVWNERKKAVSSDYDFLANLSEYYNMPN  
 LASGQFGFSSNGAGEELDYTVNESIEKGYLVLVLSYSIFGGISFAIEKR

GBS 361 may contain an N-terminal leader or signal sequence region which is indicated by  
 the underlined sequence at the beginning of SEQ ID NO: 45 above. In one embodiment, one or more  
 5 amino acids from the leader or signal sequence region are removed from GBS 361. An example of  
 such a GBS 361 fragment is set forth below as SEQ ID NO: 46.

#### SEQ ID NO: 46

VSGIGIISLGLKKNYSEHKQHLFDLKEGISKHLKYNHDSILESITGSITSDPEVPEQYKDETRNFKFAF  
 10 TAFEEALASSGVNLKAYHNIAVCLGTSLGKKSAGQNALYQFEEGERQVDASLLEKASVYHIADELMAY  
 HDIVGASYVISTACSASNNVILGTQLLDGDCDLAICGGCDELSDISLAGFTSLGAINTEMACQPYS  
 SGKGINLGEAGFVVLVKDQSLAKYKGIIGGLITSDGYHITAPKPTGEGAAQIAKQLVTQAGIDYSEI  
 DYINGHGTGTQANDKMEKNMYGKFFPTTTLISSTKGQTGHTLGAAGIIELINCLAAIEEQTVPATKNE  
 IGIEGFPENFVYHOKREYPIRNALNFSFAFGNNSGVLLSSLDSPLETLTPARENLMKMAILSSVASISK  
 15 NESLSITYEKVASNFNDFEALRFKGRPPKTVNPAQFRKMDDFSKMVAVTTAQALIESNINLKKQDTS  
 KVGIVFTTSLSGPVEVEGIEKQITTEGYAHVSASRFPFTVMNAAAGMLSIIFKITGPLSVISTNSGAL  
 DGIQYAKEMMRNDNLDYVILVSNQWTDMSFMWWQQLNYDSQMFVGS DYCSAQVLSRQALDNSPIILG  
 SKQLKYSHKTFDVTMTIFDAALQNLLSDLGLTIKDIKGFVWNERKKAVSSDYDFLANLSEYYNMPNLA  
 20 SGQFGFSSNGAGEELDYTVNESIEKGYLVLVLSYSIFGGISFAIEKR

#### GBS 404

Nucleotide and amino acid sequences of GBS 404 sequenced from serotype V isolated strain  
 2603 V/R are set forth in Ref. 2 as SEQ ID 8799 and SEQ ID 8800. These sequences are set forth  
 below as SEQ ID NOS 47 and 48:

#### SEQ ID NO. 47

ATGAAAATAGATGACCTAAGAAAAAGCGACAATGTTGAAGATCGTCGCTCCAGTAGCGGAGGTTTCATT  
 CTCTAGCGGAGGAAGTGGATTACCGATTCTTCAACTTTTATTGCTGCGAGGGAGTTTGAAAACCAAGC  
 30 TTGTGGTTTTTAATCATCTTACTGCTACTTGGCGGAGGGGGACTAACCAGCATTTTTAAATGACTCATCC  
 TCACCTTCTAGTTACCAATCTCAGAATGTCTCACGTTCTGTTGATAATAGCGCAACGAGAGAACAAAT  
 CGATTTCTGTTAATAAAGTCCTTGGCTCAACTGAGGATTTCTGGTCACAAGAATTCCAAACCCAAGGTT  
 TTGGAATTATAAGGAACCAAACTTGTCTTTACACCAATTCAATTCAAACAGGTTGTGGTATAGGT  
 GAATCTGCTTCAGGACCATTTTATTGTTGAGCAGATAAAAAAATCTATCTTGATATTTCTTTTTACAA  
 35 TGAATTATCACATAAATATGGTGCTACTGGTGATTTTGCTATGGCCTACGTCATCGCCACGAAGTTG  
 GTCACCACATTCAAACAGAGTTAGGCATTATGGATAAGTATAATAGAATGCGACACGGACTTACTAAG  
 AAAGAAGCAAATGCTTTAAATGTTGCGCTAGAACTTCAAGCAGATTATTATGCAGGGGTATGGGCTCA  
 CTACATCAGGGGAAAAAATCTCTTAGAACAAAGGAGACTTTGAAGAGGCCATGAATGCTGCCACGCCG  
 TCGGAGACGATACCCTTCAGAAAGAAACCTACGAAAATTAGTGCTGATAGCTTTACCCATGGAACA  
 40 GCTGAACAACGCCAACGTTGGTTTAAACAAAGGCTTTCAATATGGTGACATCCAACACGGTGATACTTT  
 CTCCGTAGAACATCTA

#### SEQ ID NO. 48

MKIDDLRKSDNVEDRRSSSGGSFSSGGSGLPILQLLLLRGSWKTKLVVLIILLLLGGGGLTSIFNDSS  
 SPSSYQSQNVSRVDSATREQIDFVNKVLGSTEDFWSQEFQTQGFQNYKEPKLVLYTNSIQTGCGIG  
 45 ESASGPFYCSADKKIYLDISFYNELSHKYGATGDFAMAYVIAHEVGHHIQTELGIMDKYNRMHRGLTK  
 KEANALNVRLELQADYYAGVWAHYIRGNLLEQGD FEEAMNAAHAVGDDTLQKETYGKLPVDSFTHGT  
 AEQRQRWFNKGFGYQYGD IQHGD TFSVEHL

GBS 690

Nucleotide and amino acid sequences of GBS 690 sequenced from serotype V isolated strain 2603 V/R are set forth in Ref. 2 as SEQ ID 9965 and SEQ ID 9966. These sequences are set forth as  
 5 SEQ ID NOS 49 and 50 below:

**SEQ ID NO. 49**

ATGAGTAAACGACAAAATTTAGGAATTAGTAAAAAAGGAGCAATTATATCAGGGCTCTCAGTGGCACT  
 AATTGTAGTAATAGGTGGCTTTTTATGGGTACAATCTCAACCTAATAAGAGTGCAGTAAAACTAACT  
 10 ACAAAGTTTTTAATGTTAGAGAAGGAAGTGTTCGTCCTCAACTCTTTTGACAGGAAAAGCTAAGGCT  
 AATCAAGAACAGTATGTGTATTTGATGCTAATAAAGGTAATCGAGCAACTGTCACAGTTAAAGTGGG  
 TGATAAAATCACAGCTGGTCAGCAGTTAGTTCAATATGATACAACAACTGCACAAGCAGCCTACGACA  
 CTGCTAATCGTCAATTAAATAAAGTAGCGCGTCAGATTAATAATCTAAAGACAACAGGAAGTCTTCCA  
 15 GCTATGGAATCAAGTGATCAATCTTCTTCATCATCACAAGGACAAGGGACTCAATCGACTAGTGGTGC  
 GACGAATCGTCTACAGCAAAATTATCAAAGTCAAGCTAATGCTTCATACAACCAACAACCTCAAGATT  
 TGAATGATGCTTATGCAGATGCACAGGCAGAAGTAAATAAAGCACAAAAAGCATTGAATGATACTGTT  
 ATTACAAGTGACGTATCAGGGACAGTTGTTGAAGTTAATAGTGATATTGATCCAGCTTCAAAAACCTAG  
 TCAAGTACTTGTCCATGTAGCAACTGAAGGTAACCTCAAGTACAAGGAACGATGAGTGAGTATGATT  
 TGGCTAATGTTAAAAAAGACCAGGCTGTTAAAAATAAACTAAGGTCTATCCTGACAAGGAATGGGAA  
 20 GGTAAAATTTCATATATCTCAAATTATCCAGAAGCAGAAGCAAACAACAAATGACTCTAATAACGGCTC  
 TAGTGCTGTAAATTATAAATAAAGTAGATATTACTAGCCCTCTCGATGCATTAAAAACAAGGTTTTTA  
 CCGTATCAGTTGAAGTAGTTAATGGAGATAAGCACCTTATTGTCCCTACAAGTTCTGTGATAAACAAA  
 GATAATAAACACTTTGTTTTGGGTATACAATGATTCTAATCGTAAAATTTCCAAAGTTGAAGTCAAAT  
 TGGTAAAGCTGATGCTAAGACACAAGAAATTTTATCAGGTTTGAAAGCAGGACAAATCGTGGTTACTA  
 25 ATCCAAGTAAACCTTCAAGGATGGGCAAAAAATTGATAATATTGAATCAATCGATCTTAACTCTAAT  
 AAGAAATCAGAGGTGAAA

**SEQ ID NO. 50**

MSKRQNLGISKKGAIISGLSVALIVVIGGFLWVQSQPNKSAVKTNKYKVFNVREGSVSSSTLLTGKAKA  
 30 NQEQQYVYFDANKGNRATVTVKVGDKITAGQQLVQYDTTTAQAAYDTANRQLNKVARQINNLTGSLP  
AMESSDQSSSSSQGGTQSTSGATNRLQQNYQSQANASYNQQLQDLNDAYADAQAEVNKAQKALNDTV  
ITSDVSGTVVEVNSDIDPASKTSQVLVHVATEGKLQVQGTMSSEYDLANVKKDQAVKIKSKVYPDKEWE  
GKISYISNYPEAEANNNDSSNGSSAVNYKYKVDITSPLDALKQGFTVSVEVVNGDKHLIVPTSSVINK  
 35 DNKHFWVYNDNSNRKISKVEVKIGKADAKTQEILSGLKAGQIVVTNPSKTFKDGQKIDNIESIDLNSN  
KKSEVK

GBS 690 contains an N-terminal leader or signal sequence region which is indicated by the underlined sequence at the beginning of SEQ ID NO: 50 above. In one embodiment, one or more amino acids from the leader or signal sequence region of GBS 690 are removed. An example of such  
 40 a GBS 690 fragment is set forth below as SEQ ID NO: 51.

**SEQ ID NO: 51**

FLWVQSQPNKSAVKTNKYKVFNVREGSVSSSTLLTGKAKANQEQQYVYFDANKGNRATVTVKVGDKITAG  
 45 QQLVQYDTTTAQAAYDTANRQLNKVARQINNLTGSLPAMESSDQSSSSSQGGTQSTSGATNRLQQ  
 NYQSQANASYNQQLQDLNDAYADAQAEVNKAQKALNDTVITSDVSGTVVEVNSDIDPASKTSQVLVHV  
 ATEGKLQVQGTMSSEYDLANVKKDQAVKIKSKVYPDKEWEGKISYISNYPEAEANNNDSSNGSSAVNYK  
 YKVDITSPLDALKQGFTVSVEVVNGDKHLIVPTSSVINKDNKHFWVYNDNSNRKISKVEVKIGKADAK  
 TQEILSGLKAGQIVVTNPSKTFKDGQKIDNIESIDLNSNKKSEVK

GBS 691

GBS 691 refers to an iron compound ABC transporter, or a substrate binding protein.

Nucleotide and amino acid sequences of GBS 691 sequenced from serotype V isolated strain 2603

5 V/R are set forth in Ref. 2 as SEQ ID 3691 and SEQ ID 3692. These sequences are set forth as SEQ ID NOS 52 and 53 below:

**SEQ ID NO. 52**

ATGAAAAAATTGGAATTATTGTCCTCACACTACTGACCTTCTTTTTGGTATCTTGCGGACAACAAAC  
 10 TAAACAAGAAAGCACTAAAACAACCTATTTCTAAAATGCCTAAAATTGAAGGCTTCACCTATTATGGAA  
 AAATTCTTGAAAATCCGAAAAAAGTAATTAATTTTACATATTCTTACACTGGGTATTTATTAAACTA  
 GGTGTTAATGTTTCAAGTTACAGTTTAGACTTAGAAAAAGATAGCCCCGTTTTTGGTAAACAACCTGAA  
 AGAAGCTAAAAAATTAAGTCTGATGATACAGAAGCTATTGCCGCACAAAAACCTGATTTAATCATGG  
 TTTTCGATCAAGATCCAAACATCAATACTCTGAAAAAATTCACCAACTTTAGTTATTAAATATGGT  
 15 GCACAAAATTATTAGATATGATGCCAGCCTTGGGGAAAGTATTCCGGTAAAGAAAAAGAAGCTAATCA  
 GTGGGTTAGCCAATGGAAAACTAAACTCTCGCTGTCAAAAAAGATTTACACCATATCTTAAAGCCTA  
 AACTACTTTTACTATTATGGATTTTTATGATAAAAATATCTATTTATATGGTAATAATTTTGGACGC  
 GGTGGAGAACTAATCTATGATTCAGTGGTTATGCTGCCCCAGAAAAAGTCAAAAAAGATGCTTTTAA  
 AAAAGGGTGGTTTACCGTTTCGCAAGAAGCAATCGGTGATTACGTTGGAGATTATGCCCTTGTTAATA  
 TAAACAAAACGACTAAAAAAGCAGCTTCATCACTTAAAGAAAGTGATGTCTGGAAGAATTTACCAGCT  
 20 GTCAAAAAAGGGCACATCATAGAAAGTAAGTACGACGTGTTTTATTTCTCTGACCTCTATCTTTAGA  
 AGCTCAATTAAATCATTTACAAAGGCTATCAAAGAAATACAAAT

**SEQ ID NO. 53**

MKKIGIIVLTLLTFFLVSCGQQTQESTKTTISKMPKIEGFTYYGKIPENPKKVINFTYSYTGYLELLKL  
 25 GNVNSSSYSLDLEKDSPVFGKQLKEAKKLTADDTEAIAAQKPDLMVFDQDPNINTLKKIAPTLVIKYG  
 AQNYLDMMPALGKVFGKEKEANQWVSQWKTCTLAVKKDLHHILKPNTTFTIMDFYDKNIYLYGNFGR  
 GGELIYDSLGYAAPEKVKKDVFKKGWFTVSQEAIGDYVDYALVNINKTTKKAASSLKESDVWKNLPA  
 VKKGHIIESNYDVFFYFSDPLSLEAQLKSFTKAIKENTN

30 GBS 691 contains an N-terminal leader or signal sequence region which is indicated by the underlined sequence at the beginning of SEQ ID NO: 53 above. In one embodiment, one or more amino acids are removed from the leader or signal sequence region of GBS 691. An example of such a GBS 691 fragment is set forth below as SEQ ID NO: 54.

**SEQ ID NO: 54**

EGFTYYGKIPENPKKVINFTYSYTGYLELLKLVNVNSSSYSLDLEKDSPVFGKQLKEAKKLTADDTEAIAA  
 35 QKPDLMVFDQDPNINTLKKIAPTLVIKYGAQNYLDMMPALGKVFGKEKEANQWVSQWKTCTLAVKKD  
 LHHILKPNTTFTIMDFYDKNIYLYGNFGRGGELIYDSLGYAAPEKVKKDVFKKGWFTVSQEAIGDYV  
 40 GDYALVNINKTTKKAASSLKESDVWKNLPAVKKGHIIESNYDVFFYFSDPLSLEAQLKSFTKAIKENTN

GBS 691 contains a C-terminal transmembrane or cytoplasmic region which is indicated by the underlined sequence at the end of SEQ ID NO: 53 above. In one embodiment, one or more amino acids are removed from the transmembrane or cytoplasmic region of GBS 691. An example of such a GBS 691 fragment is set forth below as SEQ ID NO: 55.

45

**SEQ ID NO: 55**

MKKI GIIIVLTLLTFFLVSCGQQTQESTKTTISKMPKIEGFTYYGKIPENPKKVINFYTSYTGYYLLKL  
 GNVVSSYSLDLEKDSPVFGKQLKEAKKLTADDTEAIAAQKPDLMVFDQDPNINTLKKIAPTLVIKYG  
 AQNYLDMMPALGKVFGEKEANQWVSQWKTCTLAVKKDLHHILKPNTTFTIMDFYDKNIYLYGNNFGR  
 5 GGELIYDSLGYAAPEKVKKDVFKKGWFTVSQEAIGDYVGDYALVNINKTTKKAASSLKESDVWKNLPA  
 VKKGHIIESNYDVIFYSDPLSLEAQLKSFT

In one embodiment, one or more amino acids from the leader or signal sequence region and  
 one or more amino acids from the transmembrane or cytoplasmic region are removed from GBS 691.

One example of such a GBS 691 fragment is set forth below as SEQ ID NO: 56

**SEQ ID NO: 56**

EGFTYYGKIPENPKKVINFYTSYTGYYLLKLGNVVSSYSLDLEKDSPVFGKQLKEAKKLTADDTEAIAA  
 QKPDLMVFDQDPNINTLKKIAPTLVIKYGAQNYLDMMPALGKVFGEKEANQWVSQWKTCTLAVKKD  
 15 LHIIILKPNTTFTIMDFYDKNIYLYGNNFGRGGELIYDSLGYAAPEKVKKDVFKKGWFTVSQEAIGDYV  
 GDYALVNINKTTKKAASSLKESDVWKNLPAVKKGHIIESNYDVIFYSDPLSLEAQLKSFT

Additional examples of GBS antigens which may be used in combination with GBS 80 are set  
 forth below.

**GBS 4**

GBS 4 refers to another putative cell wall surface anchor family protein. Nucleotide and  
 amino acid sequences of GBS 4 sequenced from serotype V isolated strain 2603 V/R are set forth in  
 Ref. 2 as SEQ ID 1 and SEQ ID 2. These sequences are also set forth below as SEQ ID NOS 57 and  
 58:

**SEQ ID NO. 57**

ATGAAAGTGAAAAATAAGATTTTAACGATGGTAGCACTTACTGTCTTAACATGTGCTACTTATTCATC  
 AATCGGTTATGCTGATACAAAGTGATAAGAATACTGACACGAGTGTCGTGACTACGACCTTATCTGAGG  
 AGAAAAGATCAGATGAAC TAGACCACTAGTACTGGTTCTTCTTCTGAAAATGAATCGAGTTCATCA  
 30 AGTGAACCAGAAACAAATCCGTCAACTAATCCACCTACAACAGAACCATCGCAACCCTCACCTAGTGA  
 AGAGAACAAAGCCTGATGGTAGAACGAAGACAGAAATTGGCAATAATAAGGATATTTCTAGTGGAACAA  
 AAGTATTAATTTCAGAAGATAGTATTAAGAATTTTAGTAAAGCAAGTAGTGATCAAGAAGAAGTGGAT  
 CGCGATGAATCATCATCTTCAAAAGCAAATGATGGGAAAAAAGGCCACAGTAAGCCTAAAAAGGAACT  
 TCCTAAACAGGAGATAGCCACTCAGATACTGTAATAGCATCTACGGGAGGGATTATTCTGTTATCAT  
 35 TAAGTTTTTACAATAAGAAAATGAACTTTAT

**SEQ ID NO. 58**

MKVKNKILTMVALTVLTCATYSSIGYADTSDKNTDTSVVTTLSEEKRSDELDQSSTGSSSENESSSS  
SEPETNPSTNPPTTEPSQPSPSEENKPDGRKTKEIGNNKDISSGTVLISEDSEIKNFSKASSDQEEVD  
 40 RDESSSSKANDGKKGHSPKKELPKTGDSHSDTVIASTGGIILLSLSFYNKMKLY

GBS 4 contains an N-terminal leader or signal sequence which is underlined at the beginning  
 of SEQ ID NO: 58 above. In one embodiment, one or more amino acids from the N-terminal leader  
 or signal peptide domain of GBS 4 are removed. An example of such a GBS 4 fragment is set forth  
 45 below as SEQ ID NO 59.

**SEQ ID NO 59**

DTSDKNTDTSVVTTLSEEKRSDELDQSSTGSSSENESSSSSEPETNPSTNPPTTEPSQSPSEENKP  
DGR TKTEIGNNKDISSG TKVLISED S IKNFSKASSDQEEVDRDESSSSKANDGKKGH SKPKKELPKTG  
DSHSDTVIASTGGIILLSLSFYNKKMKLY

5

A further N-terminal section of GBS 4 may be removed to facilitate recombinant expression.  
An example of such a GBS 4 fragment is set forth below as SEQ ID NO: 60.

**SEQ ID NO: 60**

10 DQSSTGSSSENESSSSSEPETNPSTNPPTTEPSQSPSEENKPDGR TKTEIGNNKDISSG TKVLISED  
S IKNFSKASSDQEEVDRDESSSSKANDGKKGH SKPKKELPKTGDSHSDTVIASTGGIILLSLSFYNKK  
MKLY

GBS 4 contains an C-terminal transmembrane region which is underlined at the end of SEQ  
15 ID NO: 58 above. In one embodiment, one or more amino acids from the C-terminal transmembrane  
region is removed. An example of such a GBS 4 fragment is set forth below as SEQ ID NO: 61.

**SEQ ID NO: 61**

20 MKVKNKILTMVALTVLTCATYSSIGYADTSDKNTDTSVVTTLSEEKRSDELDQSSTGSSSENESSSS  
SEPETNPSTNPPTTEPSQSPSEENKPDGR TKTEIGNNKDISSG TKVLISED S IKNFSKASSDQEEVD  
RDESSSSKANDGKKGH SKPKKE

In one embodiment, both the N-terminal leader or signal domain and the C-terminal  
transmembrane domain are removed from the GBS 4 sequence. An example of such a GBS 4  
25 fragment is set forth below as SEQ ID NO: 62.

**SEQ ID NO: 62**

30 DTSDKNTDTSVVTTLSEEKRSDELDQSSTGSSSENESSSSSEPETNPSTNPPTTEPSQSPSEENKP  
DGR TKTEIGNNKDISSG TKVLISED S IKNFSKASSDQEEVDRDESSSSKANDGKKGH SKPKKE

In yet another embodiment, the N-terminal leader or signal domain, a further N-terminal  
region and the C-terminal transmembrane domain are removed from the GBS 4 sequence. An  
example of such a GBS 4 fragment is set forth below as SEQ ID NO: 63.

**SEQ ID NO: 63**

35 DQSSTGSSSENESSSSSEPETNPSTNPPTTEPSQSPSEENKPDGR TKTEIGNNKDISSG TKVLISED  
S IKNFSKASSDQEEVDRDESSSSKANDGKKGH SKPKKE

**GBS 22**

40 GBS 22 refers to a putative adhesion lipoprotein. Nucleotide and amino acid sequences of  
GBS 22 sequenced from serotype V isolated strain 2603 V/R are set forth in Ref. 2 as SEQ 8583 and  
SEQ ID 8584. These sequences are set forth below as SEQ ID NOS 64 and 65:

**SEQ ID NO. 64**

ATGAAAAGGATACGGAAAAGCCTTATTTTTGTTCTCGGAGTAGTTACCCTAATTGCTTATGTGCTTG  
 TACTAAACAAAGCCAGCAAAAAAATGGCTTGTGAGTAGTACTAGCTTTTATCCAGTATATCCATTA  
 CAAAAGCAGTTTCTGGTGATTTGAATGATATTAATGATTCGATCACAGTCAGGTATTCATGGTTTT  
 5 GAACCCTCATCAAGTGATGTTGCTGCCATTTATGATGCTGATCTATTTCTTTATCATTTCGCACACACT  
 AGAAGCTTGGGCGAGACGTTTGAACCTAGTTTGCATCACTCTAAAGTATCTGTAATTGAAGCTTCAA  
 AAGGTATGACTTTGGATAAAGTTCATGGCTTAGAAGATGTAGAGGCAGAAAAAGGAGTAGATGAGTCA  
 ACCTTGTATGACCCTCACACTTGAATGACCCTGTAAAAGTATCTGAGGAAGCACAACTCATCGCTAC  
 ACAATTAGCTAAAAAGGATCCTAAAAACGCTAAGGTTTATCAAAAAAATGCTGATCAATTTAGTGACA  
 10 AGGCAATGGCTATTGCAGAGAAGTATAAGCCAAAATTTAAAGCTGCAAAGTCTAAATACTTTGTGACT  
 TCACATACAGCATTCTCATACTTAGCTAAGCGATACGGATTGACTCAGTTAGGTATTGCAGGTGTCTC  
 AACC GAGCAAGAACCTAGTGCTAAAAAATTAGCCGAAATTCAGGAGTTTGTGAAAACATATAAGGTTA  
 AGACTATTTTTGTTGAAGAAGGAGTCTCACCTAAATTAGCTCAAGCAGTAGCTTCAGCTACTCGAGTT  
 AAAATTGCAAGTTTAAAGTCCTTTARAAGCAGTTCCCAAAAACAATAAAGATTACTTAGAAAATTTGGA  
 15 AACTAATCTTAAGGTACTTGTCAAATCGTTAAATCAATAG

**SEQ ID NO. 65**

MKRIRKSLIFVLGVVTLICLCACTKQSQQKNGLSVVTSFYFPVYSITKAVSGDLNDIKMIRSQSGIHGF  
 EPSS SDVAAYDADLFYHSHLEAWARRLEPSLHHSKVSVEASKGMTLDKVGLEDVEAEKGVDES  
 20 TLYDPHTWNDPVKVSEEAQLIATQLAKKDPKNAKVYQKNADQFSDKAMAI AEKYKPKFKA AKSKYFVT  
 SHTA FSYLAKRYGLTQLGIAGVSTEQEPSAKKLAEIQEFVKTYKVKTIFVEEGVSPKLAQAVASATRV  
 KIASLSPLXAVPKNNKDYLENLETNLKVLVKS LNQ

**GBS 85**

25 GBS 85 refers to a putative cell division protein (DivIB). Nucleotide and amino acid  
 sequences of GBS 85 sequenced from serotype V isolated strain 2603 V/R are set forth in Ref. 2 as  
 SEQ ID 215 and SEQ ID 216. These sequences are set forth below as SEQ ID NOS 66 and 67:

**SEQ ID NO. 66**

30 ATGCCTAAGAAGAAATCAGATACCCCAGAAAAAGAAGAAGTTGTCTTAACGGAATGGCAAAAGCGTAA  
 CCTTGAATTTTTTAAAAAACGCAAAGAAGATGAAGAAGAACAAAAACGTATTAAACGAAAAATTACGCT  
 TAGATAAAAGAAGTAAATTAAATATTTCTTCTCCTGAAGAACCTCAAAATACTACTAAAATTAAGAAG  
 CTTCATTTTTCAAAGATTTCAAGACCTAAGATTGAAAAGAAACAGAAAAAAGAAAAATAGTCAACAG  
 35 CTTAGCCAAAATAATCGCATTAGAACTGCACCTATATTTGTAGTAGCATTCTAGTCATTTTAGTTT  
 CCGTTTTCTACTAACTCCTTTTAGTAAGCAAAAAACAATAACAGTTAGTGGAATCAGCATAACCT  
 GATGATATTTTGATAGAGAAAACGAATATTCAAAAAACGATTATTTCTTTCTTTAATTTTTTAAACA  
 TAAAGCTATTGAACAACGTTTAGCTGCAGAAGATGTATGGGTAAAACAGCTCAGATGACTTATCAAT  
 TTCCCAATAAGTTTCATATTCAAGTTCAAGAAAATAAGATTATTGCATATGCACATACAAAGCAAGGA  
 40 TATCAACCTGTCTTGGAACTGGAAAAAGGCTGATCCTGTAAATAGTTCAGAGCTACCAAAGCACTT  
 CTTAAACAATTAACCTTGATAAGGAAGATAGTATTAAGCTATTAATTAAGATTAAAGGCTTTAGACC  
 CTGATTTAATAAGTGAGATTCAAGGTGATAAGTTTAGCTGATTCTAAAACGACACCTGACCTCCTGCTG  
 TTAGATATGCACGATGGAAATAGTATTAGAATACCATTATCTAAATTTAAAGAAAGACTTCCTTTTAA  
 CAAACAAATTAAGAAGAACCTTAAGGAACCTTCTATTGTTGATATGGAAGTGGGAGTTTACACAACAA  
 CAAATACCATTGAATCAACCCCTGTTAAAGCAGAAGATACAAAAATAAATCAACTGATAAAACACAA  
 45 ACACAAAATGGTCAGGTGCGGAAAATAGTCAAGGACAAACAAATAACTCAAATACTAATCAACAAGG  
 ACAACAGATAGCAACAGAGCAGGCACCTAACCTCAAAATGTTAAT

**SEQ ID NO. 67**

50 MPKKKSDTPEKEEVVLTEWQKRNLEFLKKRKEDEEEQKRINEKLRLDKRSKLNISPEEPQNTTKIKK  
 LHFPKISRPKIEKKQKKEKIVNSLAKTNRI RTAPIFVVAFLVILVSVFLLTPFSKQKTITVSGNQHTP  
 DDILIEKTNIQKNDYFFSLIFKHKAIEQRLAAEDVWVKTAQMTYQFPNKFHIVQVQENKIIAYAHKQGG

5      GBS 147

10      **SEQ ID NO. 68**

-38-

CTTATCGTAAAAATTTCCATAATAATCCAAAGCAAAGTGATGGTCATTATCGTATGGATGCTCTTCAG  
 TGGAGTGGTTTAGATAAGGATGGCAAAGTTGTAGCAGATGGTTTTTATACTTATCGCTTACGTTACAC  
 ACCAGTAGCAGAAGGAGCAAATAGTCAGGAGTCAGACTTTAAAGTACAAGTAAGTACTAAGTCACCAA  
 ATCTTCCTTCACGAGCTCAGTTTGATGAACTAATCGAACATTAAGCTTAGCCATGCCTAAGGAAAGT  
 5 AGTTATGTTCTACATATCGTTTACAATTAGTTTATCTCATGTTGTAAAAGATGAAGAATATGGGGA  
 TGAGACTTCTTACCATTATTTCCATATAGATCAAGAAGGTAAAGTGACACTTCTTAAAACGGTTAAGA  
 TAGGAGAGAGTGAGGTTGCGGTAGACCCTAAGGCCTTGACACTTGTGTGGAAGATAAAGCTGGTAAT  
 TTCGCAACGGTAAAATTGTCTGATCTCTTGAATAAGGCAGTAGTATCAGAGAAAAGAAAACGCTATAGT  
 AATTTCTAACAGTTTCAAATATTTTGATAACTTGAAAAAGAACCTATGTTTTATTTCTAAAAAAGAAA  
 10 AAGTAGTAAACAAGAATCTAGAAGAAATAATATTAGTTAAGCCGCAAACCTACAGTTACTACTCAATCA  
 TTGTCTAAAGAAATAACTAAATCAGGAAATGAGAAAGTCCTCACTTCTACAAACAATAATAGTAGCAG  
 AGTAGCTAAGATCATATCACCTAAACATAACGGGGATTCTGTTAACCATACTTACCTAGTACATCAG  
 ATAGAGCAACGAATGGTCTATTTGTTGGTACTTTGGCATTGTTATCTAGTTTACTTCTTTATTTGAAA  
 CCCAAAAAGACTAAAAATAATAGTAAA

**SEQ ID NO. 69**

VDKHHSKKAILKLTLLITTSILLMHSNQVNAEEQELKNQEQSPVIANVAQQPSPSVTTNTVEKTSVTAA  
 SASNTAKEMGDTSVKNDKTEDELLEELSKNLDTSNLGADLEEEYPSKPEPTNNKESNVVTNASTAIAQ  
 KVPSAYEEVKPESKSSLAFLDTSKITKLQAITQRGKGNVVAIIDTGFDINHDI FRLDSPKDDKHSFKT  
 20 KTEFEELKAKHNITYGKWVNDKIVFAHNYANNTETVADIAAAMKDGYGSEAKNISHGTHVAGIFVGNS  
 KRPAINGLLLEGAAPNAQVLLMRI PDKIDSDFGEAYAKAITDAVNLGAKTINMSIGKTADSLIALND  
 KVKLALKLASEKGVAVVVAAGNEGAFGMDYSKPLSTNPDYGTVNSPAISEDTLVSASYESLKTISEVV  
 ETTIEGKLVKLPIVTSKPFDKGKAYDVVYANYGAKKDFEGKDFKGKIALIERGGGLDFMTKI THATNA  
 GVVGIVIFNDQEKRGNFLLIPYRELPGVGIISKVDGERIKNTSSQLTFNQSFVVDSQGGNRMLEQSSWG  
 25 VTAEGAIPDVTASGFEIYSSTYNNQYQTMSTSMASPHVAGLMTMLQSHLAEKYKGMNLD SKKLLEL  
 SKNILMSSATALYSEEDKAFYSPRQQGAGVVDAAEKAIQAQYYITGNDGKAKINLKRMDKDFDITVTIH  
 KLVEGVKELYYQANVATEQVNKGKFKALPQALLDNTWQKVLIRDKETQVRFTIDASQFSQKLKEQMAN  
 GYFLEGFVR FKEAKDSNQELMSIPFVGFGNDFANLQALETPIYKTL SKGSFYYPKNDTTHKDQLEYNE  
 SAPFESNNYTALLTQSASWGYVDYVKNNGGELELAPESPKRIILGT FENKVEDKTIHLLERDAANNPYF  
 30 AISPNKDGNRDEITPQATFLRNVKDISAQVLDQNGNVIWQSKVLPSYRKNFHNPNKQSDGHRMDALQ  
 WSGLDKDGKVADGFYTYRLRYTPVAEGANSQESDFKVQVSTKSPNLPSRAQFDETNRTL SLAMPKES  
 SYVPTYRLQLVL SHVVKDEEYGDETS YHYFHIDQEGKVTLPKTVKIGESEVAVDPKALTLVVEDKAGN  
 FATVKLSDL LNKA VVSEKENAIVISNSFKYFDNLKKEPMFISKKEKVVNKNLEEIILVKPQTTVTTQS  
 35 LSKEITKSGNEKVLSTNNNSSRVAKIISPKNHNGDSVNHTLPSTSDRATNGLFVGTLALLSSLLLYLK  
PKKTKNN SK

GBS 147 contains an N-terminal leader or signal sequence region which is indicated by the  
 underlined sequence at the beginning of SEQ ID NO 69 above. In one embodiment, one or more  
 amino acids from the leader or signal sequence region of GBS 147 are removed. An example of such  
 40 a GBS 147 fragment is set forth below as SEQ ID NO: 70.

**SEQ ID NO: 70**

EEQELKNQEQSPVIANVAQQPSPSVTTNTVEKTSVTAA SASNTAKEMGDTSVKNDKTEDELLEELSKN  
 LDTSNLGADLEEEYPSKPEPTNNKESNVVTNASTAIAQKVPSAYEEVKPESKSSLAFLDTSKITKLQAI  
 45 ITQRGKGNVVAIIDTGFDINHDI FRLDSPKDDKHSFKTKTEFEELKAKHNITYGKWVNDKIVFAHNYA  
 NNTETVADIAAAMKDGYGSEAKNISHGTHVAGIFVGNSKRPAINGLLLEGAAPNAQVLLMRI PDKIDS  
 DKFGEAYAKAITDAVNLGAKTINMSIGKTADSLIALNDKVKLALKLASEKGVAVVVAAGNEGAFGMDY  
 SKPLSTNPDYGTVNSPAISEDTLVSASYESLKTISEVVETTIEGKLVKLPIVTSKPFDKGKAYDVVYA  
 NYGAKKDFEGKDFKGKIALIERGGGLDFMTKI THATNAGVVGIVIFNDQEKRGNFLLIPYRELPGVGIIS  
 50 KVDGERIKNTSSQLTFNQSFVVDSQGGNRMLEQSSWGVTAEGAIPDVTASGFEIYSSTYNNQYQTM  
 SGTSMASPHVAGLMTMLQSHLAEKYKGMNLD SKKLLELSKNILMSSATALYSEEDKAFYSPRQQGAGV



VDAEKAIQAQYYITGNDGKAKINLKRMDGDKFDITVTIHKLVEGVKELYYQANVATEQVNGKGFALKPQ  
 ALLDTNWQKVILRDKETQVRFTIDASQFSQKLKEQMANGYFLEGFVRFKEAKDSNQELMSIPFVGFGNG  
 DFANLQALETPITYKTLKSGSFYYKPNDDTHKDQLEYNESAPFESNNYTALLTQSASWGYVDYVKNNGGE  
 LELAPESPKRIILGTFFENKVEDKTIHLLERDAANNPYFAISPNDKGNRDEITPQATFLRNVKDISAQV  
 5 LDQNGNVIWQSKVLPSYRKNFHNPNKQSDGHRMDALQWSGLDKDGKVVADGFFTYRLRYTPVAEGAN  
 SQESDFKVQVSTKSPNLPSRAQFDETNRTLSLAMPKESYVPTYRLQLVLSHVVKDEEYGDTSYHYF  
 HIDQEGKVTLPKTVKIGESEVAVDPKALTLLVVEDKAGNFATVKLSDDLKAVVSEKENAIVISNSFKY  
 FDNLKKPEPMFISKKEKVVNKNLEEIIILVKPQTTVTTQSLSKETKSGNEKVLSTNNNSSRVAKIISP  
 KHNGDSVNHTLPSTSDRATNGLFVGTLALLSSLLLYLKPKKTKNNK

GBS 147 also contains a C-terminal transmembrane and/or cytoplasmic region which may be  
 located within the underlined sequence near the end of SEQ ID NO: 69 above. In one embodiment,  
 one or more amino acids from the transmembrane and/or cytoplasmic region are removed. An  
 example of such a GBS 147 fragment is set forth below as SEQ ID NO: 71.

#### SEQ ID NO: 71

VDKHHS KKAAILKLTLITTSILLMHSNQVNAEEQELKNQEQSPVIANVAQQPSPSVTTNTVEKTSVTAA  
 SASNTAKEMGDTSVKNDKTEDELLEELSKNLDTSNLGADLEEEYPSKPETNNKESNVVTNASTAIAQ  
 KVPSAYEEVKPESKSSSLAVLDTSKITKLQAITQRGKGNVVAIIDTGFDINHDI FRLDSPKDDKHSFKT  
 20 KTEFEELKAKHNITYGKWVNDKIVFAHNYANNTETVADIAAAMKDGYGSEAKNISHGTHVAGIFVGNS  
 KRPAINGLLLEGAAPNAQVLLMRI PDKIDS DKGFEAYAKAITDAVNLGAKTINMSIGKTADSLIALND  
 KVKLALKLASEKGVAVVVAAGNEGAFGMDYSKPLSTNPDYGTVNSPAISEDTLVASYESLKTISEVV  
 ETTIEGKLVKLPIVTSKPFDKGKAYDVVYANYGAKKDFEGKDFKGKIALIERGGGLDFMTKITHATNA  
 GVVGIVIFNDQEKRGNFLIPYRELPGIISKVDGERIKNTSSQLTFNQSFVVDSSQGGNRMLEQSSWG  
 25 VTAEGA IKPDVTASGFEIYSSTYNNQYQTM SGTSMASPHVAGLMTMLQSHLAEKYKGMNLD SKKLEL  
 SKNILMSSATALYSEEDKAFYSRQQGAGVVD AEKAIQAQYYITGNDGKAKINLKRMDGDKFDITVTIHK  
 LVEGVKELYYQANVATEQVNGKGFALKPQALLDTNWQKVILRDKETQVRFTIDASQFSQKLKEQMAN  
 GYFLEGFVRFKEAKDSNQELMSIPFVGFGNGDFANLQALETPITYKTLKSGSFYYKPNDDTHKDQLEYNE  
 SAPFESNNYTALLTQSASWGYVDYVKNNGGELELAPESPKRIILGTFFENKVEDKTIHLLERDAANNPYF  
 30 AISPNKDGNRDEITPQATFLRNVKDISAQVLDQNGNVIWQSKVLPSYRKNFHNPNKQSDGHRMDALQ  
 WSGLDKDGKVVADGFFTYRLRYTPVAEGANSQESDFKVQVSTKSPNLPSRAQFDETNRTLSLAMPKES  
 SYVPTYRLQLVLSHVVKDEEYGDTSYHYFHIDQEGKVTLPKTVKIGESEVAVDPKALTLLVVEDKAGN  
 FATVKLSDDLKAVVSEKENAIVISNSFKYFDNLKKPEPMFISKKEKVVNKNLEEIIILVKPQTTVTTQS  
 LSKEITKSGNEKVLSTNNNSSRVAKIISP KHNGDSVNHT

In one embodiment, one or more amino acids from the leader or signal sequence region and  
 one or more amino acids from the transmembrane or cytoplasmic region are removed from the GBS  
 147 sequence. An example of such a GBS 147 fragment is set forth below as SEQ ID NO 72.

#### SEQ ID NO: 72

EEQELKNQEQSPVIANVAQQPSPSVTTNTVEKTSVTAA SASNTAKEMGDTSVKNDKTEDELLEELSKN  
 LDTSNLGADLEEEYPSKPETNNKESNVVTNASTAIAQKVPSAYEEVKPESKSSSLAVLDTSKITKLQAI  
 ITQRGKGNVVAIIDTGFDINHDI FRLDSPKDDKHSFKTKTEFEELKAKHNITYGKWVNDKIVFAHNYA  
 45 NNTETVADIAAAMKDGYGSEAKNISHGTHVAGIFVGNSKRPAINGLLLEGAAPNAQVLLMRI PDKIDS  
 DKGFEAYAKAITDAVNLGAKTINMSIGKTADSLIALNDKVKLALKLASEKGVAVVVAAGNEGAFGMDY  
 SKPLSTNPDYGTVNSPAISEDTLVASYESLKTISEVVETTIEGKLVKLPIVTSKPFDKGKAYDVVYA  
 NYGAKKDFEGKDFKGKIALIERGGGLDFMTKITHATNAGVVGIVIFNDQEKRGNFLIPYRELPGIIS  
 KVDGERIKNTSSQLTFNQSFVVDSSQGGNRMLEQSSWGVTAEGA IKPDVTASGFEIYSSTYNNQYQTM  
 SGTSMASPHVAGLMTMLQSHLAEKYKGMNLD SKKLEL SKNILMSSATALYSEEDKAFYSRQQGAGV

VDAEKAI QAQYYITGNDGKAKINLKRMDKFDITVTIHKLVEGVKELYQANVATEQVNKGKFALKPQ  
 ALLDTNWQKVILRDKETQVRFTIDASQFSQKLKEQMANGYFLEGFVRFKEAKDSNQELMSIPFVGFGNG  
 DFANLQALETPIYKTLKSGSFYYKPNDTTHKDQLEYNESAPFESNNYTALLTQSASWGYVDYVKNNGE  
 LELAPES PKRIILGTFENKVEDKTIHLLERDAANNPYFAISPKNKGNRDEITPQATFLRNVKDISAQV  
 5 LDQNGNV IWQSKVLPSYRKNFHNPKQSDGHYRMDALQWSGLDKDGKVVADGFGYTYRLRYTPVAEGAN  
 SQESDFKVQVSTKSPNLPSRAQFDETNRTLSLAMPKESSYVPTYRLQLVLSHVVKDEEYGDETSYPHYF  
 HIDQEGKVTLPKTVKIGESEVAVDPKALTLLVEDKAGNFATVKLSDLLNKAVVSEKENAIVISNSFKY  
 FDNLKKE PMFISKKEKVVNKNLEEIIILVKPQTTVTTQSLSKETKSGNEKVLSTSTNNSSSRVAKIISP  
 KHNGDSVNHT

# GBS 173

GBS 173 refers to an amidase family protein. Nucleotide and amino acid sequences of GBS  
 173 sequenced from serotype V isolated strain 2603 V/R are set forth in Ref. 2 as SEQ ID 8787 and  
 SEQ ID 8788. These sequences are set forth below as SEQ ID NOS 73 and 74:

## **SEQ ID NO. 73**

ATGAAACGTAAATACTTTATTCTTAATACGGTGACGGTTTAAACGTTAGCTGCTGCAATGAATACTAG  
 CAGTATCTATGCTAATAGTACTGAGACAAGTGCTTCAGTAGTTCCTACTACAAATACTATCGTTCAAA  
 CTAATGACAGTAATCCTACCGCAAAATTTGTATCAGAATCAGGACAATCTGTAATAGGTCAAGTAAAA  
 20 CCAGATAATTCTGCGGCGCTTACAACAGTTGACACGCCTCATCATATTTAGCTCCAGATGCTTTAAA  
 AACAACTCAATCAAGTCCTGTCTGTTGAGAGTACTTCTACTAAGTTAACTGAAGAGACTTACAAACAAA  
 AAGATGGTCAAGATTTAGCCAACATGGTGAGAAGTGGTCAAGTTACTAGTGAGGAACTCGTTAATATG  
 GCATACGATATTATTGCTAAAGAAAACCCATCTTTAAATGCAGTCATTACTACTAGACGCCAAGAAGC  
 25 TATTGAAGAGGCTAGAAAACCTTAAAGATACCAATCAGCCGTTTTTAGGTGTTCCCTTGTTAGTCAAGG  
 GGTTAGGGCACAGTATTAAAGGTGGTGAAACCAATAATGGCTTGATCTATGCAGATGGAAAAATTAGC  
 ACATTTGACAGTAGCTATGTCAAAAAATATAAAGATTTAGGATTTATTATTTTAGGACAAACGAACTT  
 TCCAGAGTATGGGTGGCGTAATATAACAGATTCTAAATTATACGGTCTAACGCATAATCCTTGGGATC  
 TTGCTCATAATGCTGGTGGCTCTTCTGGTGGAAGTGCAGCAGCCATTGCTAGCGGAATGACGCCAATT  
 30 GCTAGCGGTAGTGATGCTGGTGGTTCTATCCGTATTCATCTTCTTGGACGGGCTTGGTAGGTTTAAA  
 ACCAACAAGAGGATTGGTGAGTAATGAAAAGCCAGATTCGTATAGTACAGCAGTTCATTTTCCATTAA  
 CTAAGTCATCTAGAGACGCAGAAACATTATTAACCTTATCTAAAGAAAAGCGATCAAACGCTAGTATCA  
 GTTAATGATTTAAATCTTTACCAATTGCTTATACTTTGAAATCACCAATGGGAACAGAAGTTAGTCA  
 AGATGCTAAAAACGCTATTATGGACAACGTCACATTCCTTAAGAAAACAAGGATTCAAAGTAACAGAGA  
 35 TAGACTTACCAATTGATGGTAGAGCATTAATGCGTGATTATTCAACCTTGGCTATTGGCATGGGAGGA  
 GCTTTTTCAACAATTGAAAAAGACTTAAAAAACATGGTTTTACTAAAGAAGACGTTGATCCTATTAC  
 TTGGGCAGTTCATGTTATTTATCAAAATTCAGATAAGGCTGAACCTTAAGAAATCTATTATGGAAGCCC  
 AAAACATATGGATGATTATCGTAAGGCAATGGAGAAGCTTCACAAGCAATTTCTATTTTCTTATCG  
 CCAACGACCGCAAGTTTAGCCCCCTCTAAATACAGATCCATATGTAACAGAGGAAGATAAAAGAGCGAT  
 40 TTATAATATGGAAAACCTTGAGCCAAGAAGAAAGAAATTGCTCTCTTTAATCGCCAGTGGGAGCCTATGT  
 TGCCTAGAACACCTTTTACACAAATTGCTAATATGACAGGACTCCCAGCTATCAGTATCCCGACTTAC  
 TTATCTGAGTCTGGTTTACCCATAGGGACGATGTTAATGGCAGGTGCAAACTATGATATGGTATTAAT  
 TAAATTTGCAACTTTCTTTGAAAAACATCATGGTTTTAATGTTAAATGGCAAAGAATAATAGATAAAG  
 AAGTGAAACCATCTACTGGCCTAATACAGCCTACTAACTCCCTCTTTAAAGCTCATTCATCATTAGTA  
 45 AATTTAGAAAGAAAATTCAACAAGTTACTCAAGTATCTATCTCTAAAAAATGGATGAAATCGTCTGTAA  
 AAATAAACCATCCGTAATGGCATATCAAAAAGCACTTCCTAAAAACAGGTGATACAGAATCAAGCCTAT  
 CTCCAGTTTTAGTAGTAACCCTTTTATTAGCTTGTTTTAGCTTTGTAACAAAAAAGAATCAGAAAAGT

**SEQ ID NO. 74**

MKRKYFILNTVTVLTLAAAMNTSSIIYANSTETSASVVPTTNTIVQTNDNPTAKFVSESGQSVIGQVK  
 PDNSAALTTVDTPHHISAPDALKTTQSSPVVESTSTKLTEETYKQKDGQDLANMVRSGQVTSEELVNM  
 5 AYDIIAKENPSLNAVITTRRQEAIEEARKLKDTNQPFLLVGLKGLGHSIKGGETNNGLIYADGKIS  
 TFDSSYVKKYKDLGFIILGQTNFPEYGWRNITDSKLYGLTHNPWDLAHNAGGSSGGSAAAIASGMTPI  
 ASGSDAGGSIRIPSSWTGLVGLKPTRGLVSNEKPDSYSTAVHFPLTKSSRDAETLLTYLKKSQDTLVS  
 VNDLKSLPIAYTLKSPMGTEVSQDAKNAIMDNVTFLRKQGFVKTEIDLPIDGRALMRDYSTLAIGMGG  
 10 AFSTIEKDLKKHGFTKEDVDPI TWAVHVIYQNSDKAELKKSIMEAQKHMDYRKAMEKLHKQFPFI  
 PTTASLAPLNTDPYVTEEDKRAIYNMENLSQEERIALFNRQWEPMLRRTPTQIANMTGLPAISIPTY  
 LSESGLPIGTMLMAGANYDMVLIK FATFFEKHHGFNVKWQRIIDKEVKPSTGLIQPTNSLFKAHSSLV  
 NLEENSQVTQVSISKKWMKSSVKNKPSVMAYQKALPKTGDTESLSPLVLTLLACFSFVTKKNQKS

GBS 173 contains an N-terminal leader or signal sequence region which is indicated by the  
 15 underlined sequences at the beginning of SEQ ID NO: 74 above. In one embodiment, one or more  
 amino acids from the leader or signal sequence of GBS 173 are removed. An example of such a GBS  
 173 fragment is set forth below as SEQ ID NO: 75.

**SEQ ID NO: 75**

TTNTIVQTNDNPTAKFVSESGQSVIGQVKPDNSAALTTVDTPHHISAPDALKTTQSSPVVESTSTKL  
 20 TEETYKQKDGQDLANMVRSGQVTSEELVNMA YDIIAKENPSLNAVITTRRQEAIEEARKLKDTNQPFLL  
 GVPLLVKGLGHSIKGGETNNGLIYADGKISTFDSSYVKKYKDLGFIILGQTNFPEYGWRNITDSKLYG  
 LTHNPWDLAHNAGGSSGGSAAAIASGMTPIASGSDAGGSIRIPSSWTGLVGLKPTRGLVSNEKPDSYS  
 TAVHFPLTKSSRDAETLLTYLKKSQDTLVS VNDLKSLPIAYTLKSPMGTEVSQDAKNAIMDNVTFLRK  
 25 QGFVKTEIDLPIDGRALMRDYSTLAIGMGGAFSTIEKDLKKHGFTKEDVDPI TWAVHVIYQNSDKAEL  
 KKSIMEAQKHMDYRKAMEKLHKQFPFI FLSPPTASLAPLNTDPYVTEEDKRAIYNMENLSQEERIALF  
 NRQWEPMLRRTPTQIANMTGLPAISIPTYLSESGLPIGTMLMAGANYDMVLIK FATFFEKHHGFNVK  
 WQRIIDKEVKPSTGLIQPTNSLFKAHSSLVNLEENSQVTQVSISKKWMKSSVKNKPSVMAYQKALPKT  
 30 GDTESLSPLVLTLLACFSFVTKKNQKS

GBS 173 may also contain a C-terminal transmembrane and/or cytoplasmic region which  
 may be located within the underlined region near the end of SEQ ID NO: 74 above. In one  
 embodiment, one or more amino acids from the transmembrane or cytoplasmic region of GBS 173 are  
 removed. An example of such a GBS 173 fragment is set forth below as SEQ ID NO: 76.

**SEQ ID NO: 76**

MKRKYFILNTVTVLTLAAAMNTSSIIYANSTETSASVVPTTNTIVQTNDNPTAKFVSESGQSVIGQVK  
 PDNSAALTTVDTPHHISAPDALKTTQSSPVVESTSTKLTEETYKQKDGQDLANMVRSGQVTSEELVNM  
 40 AYDIIAKENPSLNAVITTRRQEAIEEARKLKDTNQPFLLVGLKGLGHSIKGGETNNGLIYADGKIS  
 TFDSSYVKKYKDLGFIILGQTNFPEYGWRNITDSKLYGLTHNPWDLAHNAGGSSGGSAAAIASGMTPI  
 ASGSDAGGSIRIPSSWTGLVGLKPTRGLVSNEKPDSYSTAVHFPLTKSSRDAETLLTYLKKSQDTLVS  
 VNDLKSLPIAYTLKSPMGTEVSQDAKNAIMDNVTFLRKQGFVKTEIDLPIDGRALMRDYSTLAIGMGG  
 AFSTIEKDLKKHGFTKEDVDPI TWAVHVIYQNSDKAELKKSIMEAQKHMDYRKAMEKLHKQFPFI  
 45 PTTASLAPLNTDPYVTEEDKRAIYNMENLSQEERIALFNRQWEPMLRRTPTQIANMTGLPAISIPTY  
 LSESGLPIGTMLMAGANYDMVLIK FATFFEKHHGFNVKWQRIIDKEVKPSTGLIQPTNSLFKAHSSLV  
 NLEENSQVTQVSISKKWMKSSVKNK

In one embodiment, one or more amino acids from the leader or signal sequence region and one or more amino acids from the transmembrane or cytoplasmic region are removed. An example of such a GBS 173 fragment is set forth below as SEQ ID NO: 77.

5 **SEQ ID NO: 77**

TTNTIVQTNDNSNPTAKFVSESGQSVIGQVKPDNSAALTTVDTPHHISAPDALKTTQSSPVVESTSTKL  
TEETYKQKDGQDLANMVRSGQVTSEELVNMAVDIIAKENPSLNAVITTRRQEAIEEARKLKDTNQPFLL  
GVPLLVKGLGHSIKGGETNNGLIYADGKISTFDSSYVKKYKDLGFILGQTNFPEYGWRNITDSKLYG  
LTHNPWDLAHNAGGSSGGSAAAIASGMTPIASGSDAGGSIRIPSSWTGLVGLKPTRGLVSNEKPDSYS  
10 TAVHFPLTKSSRDAETLLTYLKSDQTLVSVNDLKSLPIAYTLKSPMGTEVSQDAKNAIMDNVTFLRK  
QGFKVTEIDLPIDGRALMRDYSTLAIGMGGAFTSIEKDLKKHGFTKEDVDPITWAVHVIIYQNSDKAEL  
KKSIMEAQKHMDDYRKAMEKLHKQFFIFLSPTTASLAPLNTDPYVTEEDKRAIYNMENLSQEERIALF  
NRQWEPMLRRTPTFTQIANMTGLPAISIPTYLSESLPIGTMLMAGANYDMVLIKFAFFFEKHHGFNVK  
15 WQRIIDKEVKPSTGLIQPTNSLFKAHSSLVNLEENSQVTQVSISKKWMKSSSVKNK

15 **GBS 313**

Nucleotide and amino acid sequences of GBS 313 sequenced from serotype V isolated strain 2603 V/R are set forth in Ref. 2 as SEQ ID 4089 and SEQ ID 4090. These sequences are set forth as SEQ ID NOS 78 and 79 below:

20 **SEQ ID NO. 78**

ATGAAACGTATTGCTGTTTTAACTAGTGGTGGTGACGCCCTGGTATGAACGCTGCTATCCGTGCAGT  
TGTTCTGTAAGCAATTTCTGAAGGTATGGAAGTTTACGGCATCAACCAAGTTACTATGGTATGGTGA  
CAGGGGATATTTTCCCTTTGGATGCTAATTTCTGTTGGGGATACTATCAACCGTGGAGGAACGTTTTTA  
25 CGTTCAGCACGTTATCCTGAATTTGCTGAACCTGAAGGTCAGCTTAAAGGGATTGAACAGCTTAAAAA  
ACACGGTATTGAAGGTGTAGTAGTTATCGGTGGTGATGGTTCTTATCATGGTGCTATGCGTCTAACTG  
AGCACGGTTTCCCAGCTGTTGGTTTGCCGGGTACAATTGATAACGATATCGTTGGCACTGACTATACT  
ATTGGTTTTGACACAGCAGTTGCGACAGCAGTTGAGAATCTTGACCGTCTTCGTGATACATCAGCAAG  
TCATAACCGTACTTTTGTGTTGAGGTTATGGGAAGAAATGCAGGAGATATCGCTCTTTGGTCAGGTA  
30 TCGCTGCAGGTGCAGATCAAATTATTGTTCTGGAAGAGAGTTCAATATTGATGAAGTTGTCTCAAAT  
GTTAGAGCTGGCTATGCAGCTGGTAAACATCACCAAATCATCGTCCTTGCAAGAGGTGTTATGAGTGG  
TGATGAGTTTGCAAAAACAATGAAAGCAGCAGGAGACGATAGCGATCTTCGTGTGACGAATTTAGGAC  
ATCTGCTCCGTGGTGGTAGTCCGACGGCTCGTGATCGTGTCTTAGCATCTCGTATGGGAGCGTACGCT  
GTTCAATTGTTGAAAGAAGGTCGTGGTGGTTTAGCCGTTGGTGTCCACAACGAAGAAATGGTTGAAAG  
35 TCCAATTTTAGGTTTAGCAGAAGAAGGTGCTTTGTTGAGCTTGACTGATGAAGGAAAAATCGTTGTTA  
ATAATCCGCATAAAGCGGACCTTCGCTTGCGCAGCACTTAATCGTGACCTTGCCAACCAAGTAGTAA

40 **SEQ ID NO. 79**

MKRIAVLTSGGDAPGMNAAIRAVVRKAISEGMEVYGINQGYGMVTGDI FPLDANSVGDITINRGGTFLL  
RSARYPEFAELEGQLKGIEQLKKHGIIEGVVIGGDGSYHGAMRLTEHGFPVGLPGTIDNDIVGTDYT  
IGFDTAVATAVENLDRLRDTASASHNRTFVVEVMGRNAGDIALWSGIAAGADQII VPEEEFNIDEVVS  
VRAGYAAGKHHQII VLAEGVMSGDEFKTMKAAGDDSDLRVTNLGHLLRGSPTARDRVLASRMGAYA  
VQLLKEGRGGLAVGVHNEEMVESPIGLAEEGALFSLTDEGKIVVNNPHKADRLRLAALNRDLANQSSK

GBS 328

GBS 328 belongs to the 5'-nucleotidase family. Nucleotide and amino acid sequences of GBS 328 sequenced from serotype V isolated strain 2603 V/R are set forth in Ref. 2 as SEQ ID 6015 and SEQ ID 6016. These sequences are set forth below as SEQ ID NOS 80 and 81:

**SEQ ID NO. 80**

ATGAAAAAGAAATTATTTTAAAAAGTAGTGTCTTGGTTTAGTCGCTGGGACTTCTATTATGTTCTC  
AAGCGTGTTCGCGGACCAAGTCGGTGTCCAAGTTATAGGCGTCAATGACTTTTCATGGTGCACTTGACA  
10 ATACTGGAACAGCAAAATATGCCTGATGGAAAAGTTGCTAATGCTGGTACTGCTGCTCAATTAGATGCT  
TATATGGATGACGCTCAAAAAGATTTCAAACAACTAACCCTAATGGTGAAAGCATTAGGGTTCAAGC  
AGGCGATATGGTTGGAGCAAGTCCAGCCAACTCTGGGCTTCTTCAAGATGAACCAACTGTCAAAAATT  
TTAATGCAATGAATGTTGAGTATGGCACATTGGGTAACCATGAATTTGATGAAGGGTTGGCAGAATAT  
AATCGTATCGTTACTGGTAAAGCCCCTGCTCCAGATTCTAATATTAATAATATTACGAAATCATACCC  
15 ACATGAAGCTGCAAAACAAGAAATTGTAGTGGCAAATGTTATTGATAAAGTTAACAAACAAATTCCTT  
ACAATTGGAAGCCTTACGCTATTAAAAATATTCTGTAAATAACAAAAGTGTGAACGTTGGCTTTATC  
GGGATTGTCAACCAAAGACATCCCAAACCTTGTCTTACGTAAAAATTATGAACAATATGAATTTTTAGA  
TGAAGCTGAAACAATCGTTAAATACGCCAAAGAATTACAAGCTAAAAATGTCAAAGCTATTGTAGTTC  
TCGCACATGTACCTGCAACAAGTAAAAATGATATTGCTGAAGGTGAAGCAGCAGAAATGATGAAAAAA  
20 GTCAATCAACTCTTCCCTGAAAATAGCGTAGATATTGTCTTTGCTGGACACAATCATCAATATACAAA  
TGGTCTTGTGTGTAAGTAACTCGTATTGTACAAGCGCTCTCTCAAGGAAAAGCCTATGCTGATGTACGTG  
GTGTCTTAGATACCTGATACACAAGATTTCAATGAGACCCCTTCAGCTAAAGTAATTGCAGTTGCTCCT  
GGTAAAAAACAGGTAGTGCCGATATTCAAGCCATTGTTGACCAAGCTAATACTATCGTTAAACAAGT  
AACAGAAGCTAAATTGGTACTGCCGAGGTAAGTGTGATGATTACGCGTTCTGTTGATCAAGATAATG  
25 TTAGTCCGGTAGGCAGCCTCATCACAGAGGCTCAACTAGCAATTGCTCGAAAAAGCTGGCCAGATATC  
GATTTTGCCATGACAAATAATGGTGGCATTTCGTGCTGACTTACTCATCAAACCAGATGGAACAATCAC  
CTGGGGAGCTGCACAAGCAGTTCAACCTTTTGGTAATATCTTACAAGTCGTCGAAATTACTGGTAGAG  
ATCTTTTATAAAGCACTCAACGAACAATACGACCAAAAAACAAAATTTCTTCCTTCAAATAGCTGGTCTG  
CGATACACTTACACAGATAATAAAGAGGGCGGGGAAGAAACACCATTTAAAGTTGTAAGGCTTATAA  
30 ATCAAATGGTGAGGAAATCAATCCTGATGCAAAATACAAATTAGTTATCAATGACTTTTTTATTCGGTG  
GTGGTGATGGCTTTGCAAGCTTCAGAAATGCCAACTTCTAGGAGCCATTAACCCCGATACAGAGGTA  
TTTATGGCCTATATCACTGATTTAGAAAAAGCTGGTAAAAAAGTGAGCGTTCCAAATAATAAACCTAA  
AATCTATGTCACTATGAAGATGGTTAATGAACTATTACACAAAATGATGGTACACATAGCATTATTA  
AGAACTTTATTTAGATCGACAAGGAAATATTGTAGCACAAAGAGATTGTATCAGACACTTTAAACCAA  
35 ACAAATCAAAATCTACAAAAATCAACCCTGTAACCTACAATTCACAAAAACAATTACACCAATTTAC  
AGCTATTAACCCTATGAGAAATTATGGCAAACCATCAAACCTCACTACTGTAAAATCAAAACAATTAC  
CAAAACAACCTCTGAATATGGACAATCATTCCTTATGTCTGTCTTTGGTGTGGACTTATAGGAATT  
GCTTTAAATACAAAGAAAAACATATGAAA

**SEQ ID NO. 81**

MKKKIIILKSSVLGLVAGTSIMFSSVFADQVGVQVIGVNDFH GALDNTGTANMPDGKVANAGTAAQLDA  
YMDDAQKDFKQTNPNGESIRVQAGDMVGAS PANSGLLQDEPTVKNFNAMNVEYGT LGNHEFDEGLAEY  
NRIVTGKAPAPDSNINNITKSY PHEAAKQEIVVANVIDKVNKQI PYNWKPYAIKNI PVNNKSVNVGFI  
GIVTKDIPNLVLRKNYEQYEF LDEAETIVKYAKELQAKNVKAI VVLAHVPATSKNDIAEGEAAEMMKK  
45 VNQLFPENSVDIVFAGHNH QYTNGLVGKTRIVQALSQ GKAYADVRGVLD TDTQDFIETPSAKVIAVAP  
GKKTGSADIQAI V DQANTIVKQVTEAKI GTAEVSVMITRSVDQDNVSPV GSLITEAQ LAIARKSWPDI  
DFAMTNNGGIRADLLIKPDGTITWGAAQAVQ PFGNILQVVEITGRDLYKALNEQYDQKQNF FLQIAGL  
RYTYTDNKEGGEETPFKVVKAYKSN GEEINPD AKYKLVINDFLFGGGDGFASFRNAKLLGAINPDTEV  
FMAYITDLEKAGK KVSVPNNKPKI YVTM KVMNETITQNDGTHS I IKKLYLDRQGNIVAQEIVSDTLNQ  
50 TKSKSTKINPVTTIHKQLHQFTAINPMRNYGKPSNSTTVKSKQLPKTNSEYQGSFLMSVFGVGLIGI  
ALNTKKKHKMK

GBS 328 may contain an N-terminal leader or signal sequence region which is indicated by the underlined sequence at the beginning of SEQ ID NO: 81 above. In one embodiment, one or more amino acids from the leader or signal sequence region of GBS 328 are removed. An example of such a GBS 328 fragment is set forth below as SEQ ID NO: 82.

**SEQ ID NO: 82**

HGALDNTGTANMPDGKVANAGTAAQLDAYMDDAQKDFKQTNPNGESIRVQAGDMVGASPANSGLLQDE  
PTVKNFNAMNVEYGTGLGNHEFDEGLAEYNRIVTGKAPAPDSNINNITKSY PHEAAKQEIVVANVIDKV  
NKQIPYNWKPYAIKNI PVNNKSVNVGFIGIVTKDIPNLVLRKNYEQYEFLEAETIVKYAKELQAKNV  
KAI VVLAHV PATSKNDIAEGEAAEMMKVNQLFPENSVDIVFAGHNHQYTNGLVGKTRIVQALSQGKA  
YADV RGVLD TDTQDFIETPSAKVIAVAPGKKTGSADIQAIVDQANTIVKQVTEAKIGTAEVSVMITRS  
VDQDNVSPVGS LITEAQLAIARKSWPDI DFAMTNNGGIRADLLIKPDGTITWGAAQAVQPF GNILQVV  
EITGRDLYKALNEQYDQKQNF FLQIAGLRYTYTDNKEGGEETPFKVVKAYKSN GEEINPD AKYKLVIN  
DFLFGGGDGFASFRNAKLLGAINPDTEVF MAYITDLEKAGKKVSPNNKPKIYVTM K MVNETITQNDG  
THSIIKKLYLDRQGNIVAQEIVSDTLNQTKSKSTKINPVTTIHKKQLHQFTAINPMRNYGKPSNSTTV  
KSKQLPKTNSEYGSFLMSVFGVGLIGIALNTKKKHKM

GBS 328 may also contain a transmembrane and/or cytoplasmic domain region. In one embodiment, one or more amino acids from the transmembrane and/or cytoplasmic domain region of GBS 328 are removed. An example of such a GBS 328 fragment is set forth below as SEQ ID NO: 83.

**SEQ ID NO: 83**

MKKKIILKSSVLGLVAGTSIMFSSVFADQVGVQVIGVND F HGALDNTGTANMPDGKVANAGTAAQLDA  
YMDDAQKDFKQTNPNGESIRVQAGDMVGASPANSGLLQDEPTVKNFNAMNVEYGTGLGNHEFDEGLAEY  
NRIVTGKAPAPDSNINNITKSY PHEAAKQEIVVANVIDKVNKQIPYNWKPYAIKNI PVNNKSVNVGFI  
GIVTKDIPNLVLRKNYEQYEFLEAETIVKYAKELQAKNVKAI VVLAHV PATSKNDIAEGEAAEMMKK  
VNQLFPENSVDIVFAGHNHQYTNGLVGKTRIVQALSQGKAYADV RGVLD TDTQDFIETPSAKVIAVAP  
GKKTGSADIQAIVDQANTIVKQVTEAKIGTAEVSVMITRSVDQDNVSPVGS LITEAQLAIARKSWPDI  
DFAMTNNGGIRADLLIKPDGTITWGAAQAVQPF GNILQVVEITGRDLYKALNEQYDQKQNF FLQIAGL  
RYTYTDNKEGGEETPFKVVKAYKSN GEEINPD AKYKLVINDFLFGGGDGFASFRNAKLLGAINPDTEV  
FMAYITDLEKAGKKVSPNNKPKIYVTM K MVNETITQNDGTHSIIKKLYLDRQGNIVAQEIVSDTLNQ  
TKSKSTKINPVTTIHKKQLHQFTAINPMRNYGKPSNSTTVKS

In one embodiment, one or more amino acids from the leader or signal sequence region and one or more amino acids from the transmembrane or cytoplasmic region of GBS 328 are removed. An example of such a GBS 328 fragment is set forth below as SEQ ID NO: 84.

**SEQ ID NO: 84**

HGALDNTGTANMPDGKVANAGTAAQLDAYMDDAQKDFKQTNPNGESIRVQAGDMVGASPANSGLLQDE  
PTVKNFNAMNVEYGTGLGNHEFDEGLAEYNRIVTGKAPAPDSNINNITKSY PHEAAKQEIVVANVIDKV  
NKQIPYNWKPYAIKNI PVNNKSVNVGFIGIVTKDIPNLVLRKNYEQYEFLEAETIVKYAKELQAKNV  
KAI VVLAHV PATSKNDIAEGEAAEMMKVNQLFPENSVDIVFAGHNHQYTNGLVGKTRIVQALSQGKA  
YADV RGVLD TDTQDFIETPSAKVIAVAPGKKTGSADIQAIVDQANTIVKQVTEAKIGTAEVSVMITRS  
VDQDNVSPVGS LITEAQLAIARKSWPDI DFAMTNNGGIRADLLIKPDGTITWGAAQAVQPF GNILQVV

EI TGRDLYKALNEQYDQKQNFLLQIAGLRYTYTDNKEGG EETPFKVVKAYKSN GEEINPD AKYKLVIN  
DFLFGGGDGFASF RNAKLLGAINPDTEVF MAYITDLEKAGKKVSV PNNKPKIYVTM KMVNETITQNDG  
THSIIKKLYLDRQGNIVAQEIVSDTLNQT KSKSTKINPVT TIHKKQLHQFTAINPMRNYGKPSNSTTV  
KS

### GBS 656

GBS 656 refers to a putative DNA-entry nuclease. Nucleotide and amino acid sequences of GBS 656 sequenced from serotype V isolated strain 2603 V/R are set forth in Ref. 2 as SEQ ID 9323 and SEQ ID 9324. These sequences are set forth below as SEQ ID NOS 85 and 86:

#### **SEQ ID NO. 85**

ATGAAAAGATTACATAAACTGTTTATAACCGTAATTGCTACATTAGGTATGTTGGGGGTAATGACCTT  
TGGTCTTCCAACGCAGCCGCAAAACGTAACGCCGATAGTACATGCTGATGTCAATTCATCTGTTGATA  
CGAGCCAGGAATTTCAAATAATTTAAAAAATGCTATTGGTAACCTACCATTTCAATATGTTAATGGT  
ATTTATGAATTAAATAATAATCAGACAAATTTAAATGCTGATGTCAATGTTAAAGCGTATGTTCAAAA  
TACAATTGACAATCAACAAAGACTATCAACTGCTAATGCAATGCTTGATAGAACCATTCGTCAATATC  
AAAATCGCAGAGATACCACTCTTCCCGATGCAAATTGGAACCATTAGGTTGGCATCAAGTAGCTACT  
AATGACCATTATGGACATGCAGTCGACAAGGGGCATTTAATTGCCTATGCTTTAGCTGGAAATTTCAA  
AGGTTGGGATGCTTCCGTGTCAAATCCTCAAATGTTGTACACAAACAGCTCATTCCAACCAATCAA  
ATCAAAAAATCAATCGTGGACAAAATTATTATGAAAGCTTAGTTCGTAAGGCGGTTGACCAAAACAAA  
CGTGTTTCGTTACCGTGTAACCTCCATTGTACCGTAATGATACTGATTTAGTTCATTGCAATGCACCT  
AGAAGCTAAATCACAAGATGGCACATTAGAATTTAATGTTGCTATTCCAAACACACAAGCATCATACA  
CTATGGATTATGCAACAGGAGAAATAACACTAAAT

#### **SEQ ID NO. 86**

MKRLHKLFI TVIATLGLGVMTFGLPTQPQNVTPIVHADVNSSVDTSQEFQNNLKNAIGNLPFQYVNG  
IYELNNNQTNLNADVN VKAYVQNTIDNQQLSTANAMLDRTIRQYQNRD TTPDANWKPLGWHQVAT  
NDHYGHAVDKGHLIAYALAGNFKGWDASVSNPQNVVTQTAHSNQSNQKINRGQNYYESLVRKAVDQNK  
RVRYRVTPLYRNDTDLVPPFAMHLEAKSQDGTLEFNVAIPNTQASYTMDYATGEITLN

### GBS 67

The following offers examples of preferred GBS 67 fragments. Nucleotide and amino acid sequence of GBS 67 sequences from serotype V isolated strain 2603 are set forth below as SEQ ID NOS: 87 and 88.

#### **SEQ ID NO: 87**

ATGAGAAAATACCAAAAATTTTCTAAATATTGACGTTAAGTCTTTTTTGTGTCGCAAATACCGCT  
TAA TACCAATGTTTTAGGGGAAAGTACCGTACCGGAAATGGTGCTAAAGGAAAGTTAGTTGTTAAAA  
AGACAGATGACCAGAACAACCACTTTCAAAGCTACCTTTGTTTTAAAACTACTGCTCATCCAGAA  
AGTAAAATAGAAAAAGTAACTGCTGAGCTAACAGGTGAAGCTACTTTTGATAATCTCATACCTGGAGA  
TTA TACTTTATCAGAAGAAACAGCGCCCGAAGGTTATAAAAAGACTAACCAGACTTGGCAAGTTAAGG  
TTGAGAGTAATGGAAAACTACGATACAAAATAGTGGTGATAAAAATTCACAATTGGACAAAATCAG  
GAA GAACTAGATAAGCAGTATCCCCCACAGGAATTTATGAAGATACAAAGGAATCTTATAAACTTGA  
GCA TGTTAAAGGTTCA GTTCCAAATGGAAAGTCAGAGGCAAAAGCAGTTAACC CATATTCAAGTGAAG  
GTGAGCATATAAGAGAAATTCAGAGGGAACATTATCTAAACGTATTT CAGAAGTAGGTGATTTAGCT  
CAT AATAAATATAAAATTGAGTTAACTGT CAGTGGAAAAACCATAGTAAAACCA GTGGACAAACAAA  
GCC GTTAGATGTTGTCTTCGTACTCGATAATTCTAACTCAATGAATAACGATGGCCCAAATTTTCAA  
GGCATAATAAAGCCAAGAAAGCTGCCGAAGCTCTTGGGACCGCAGTAAAAGATATTTTAGGAGCAAAC

AGTGATAATAGGGTTGCATTAGTTACCTATGGTTCAGATATTTTTGATGGTAGGAGTGTAGATGTCGT  
 AAAAGGATTTAAAGAAGATGATAAATATTATGGCCTTCAAACCTAAGTTCACAATTCAGACAGAGAATT  
 ATAGTCATAAACAATTAACAAATAATGCTGAAGAGATTATAAAAAGGATTCGACAGAAGCTCCTAAA  
 5 GCTAAGTGGGGATCTACTACCAATGGATTAACTCCAGAGCAACAAAAGGAGTACTATCTTAGTAAAGT  
 AGGAGAAACATTTACTATGAAAGCCTTCATGGAGGCAGATGATATTTGAGTCAAGTAAATCGAAATA  
 GTCAAAAAATTATTGTTTCATGTAAGTATGGTGTTCCTACGAGATCATATGCTATTAATAATTTTAAA  
 CTGGGTGCATCATATGAAAGCCAATTTGAACAAATGAAAAAAATGGATATCTAAATAAAAGTAATTT  
 TCTACTTACTGATAAGCCCCGAGGATATAAAAGGAAATGGGGAGAGTTACTTTTTTGTTCCTTAGATA  
 10 GTTATCAAACACAGATAATCTCTGGAACTTACAAAACTTCATTATTTAGATTTAAATCTTAATTAC  
 CCTAAAGGTACAATTTATCGAAATGGACCAGTGAAAGAACATGGAACACCAACCAAACCTTTATATAAA  
 TAGTTTAAAAACAGAAAAATTATGACATTTTAAATTTTGGTATCGATATATCTGGTTTTAGACAAGTTT  
 ATAATGAGGAGTATAAGAAAAATCAAGATGGTACTTTTCAAAAATTGAAAGAGGAAGCTTTTAACTT  
 TCAGATGGAGAAATCACAGAACTAATGAGGTCGTTCTCTTCCAAACCTGAGTACTACACCCCTATCGT  
 AACTTCAGCCGATACATCTAACAATGAAATTTTATCTAAAATTCAGCAACAATTTGAAACGATTTTAA  
 15 CAAAAGAAAACCTCAATTGTTAATGGAACATCGAAGATCCTATGGGTGATAAAATCAATTTACAGCTT  
 GGTAAATGGACAAACATTACAGCCAAGTGATTATACTTTACAGGGAAATGATGGAAGTGTAATGAAGGA  
 TGGTATTGCAACTGGTGGGCCTAATAATGATGGTGGAATACTTAAGGGGGTTAAATTAGAATACATCG  
 GAAATAAACTCTATGTTAGAGGTTTGAATTTAGGAGAAGGTCAAAAAGTAACACTCACATATGATGTG  
 AAAGTAGATGACAGTTTTATAAGTAACAAATTTCTATGACACTAATGGTAGAACAACATTGAATCCTAA  
 20 GTCAGAGGATCCTAATACACTTAGAGATTTTCCAATCCCTAAAATTCGTGATGTGAGAGAATATCCTA  
 CAATAACGATTAAAAACGAGAAGAAGTTAGGTGAAATTTGAATTTATAAAAAGTTGATAAAGATAATAAT  
 AAGTTGCTTCTCAAAGGAGCTACGTTTGAACCTCAAGAATTTAATGAAGATTATAAACTTTATTTACC  
 AATAAAAAATAATAATCAAAGTAGTGACGGGAGAAAACGGCAAAATTTCTTACAAAGATTTGAAAG  
 25 ATGGCAAATATCAGTTAATAGAAGCAGTTTCGCCGGAGGATTATCAAAAAATTACTAATAAACCAATT  
 TTAACTTTTGAAGTGGTTAAAGGATCGATAAAAAATATAATAGCTGTTAATAAACAGATTTCTGAATA  
 TCATGAGGAAGGTGACAAGCATTTAATTACCAACACGCATATTCCACCAAAGGAATTATTCCTATGA  
 CAGGTGGGAAAGGAATTCTATCTTTCATTTAATAGGTGGAGCTATGATGTCTATTGCAGGTGGAATT  
 TATATTTGGAAAAGGTATAAGAAATCTAGTGATATGTCCATCAAAAAAGAT

30 **SEQ ID NO: 88**

MRKYQKFSKILTLFLSLSQIPLNTNVLGESTVPENGAAGKLVVKKTDQNKPLSKATFVLKTTAHPE  
 SKIEKVTAELTGEATFDNLIPGDYTLSEETAPEGYKKTNQWQVKVESNGKTTIQNSGDKNSTIGQONQ  
 EELDKQYPPGTGIYEDTKESYKLEHVKGSVPNGKSEAKAVNPYSSEGEHIREIPEGTLISKRISEVGDLA  
 HNKYKIELTVSGKTIVKPVDKQKPLDVVFLDNSNSMNDGPNFQRHNKAKKAAEALGTAVKDILGAN  
 35 SDNRVALVTYGSDFDGRSDVVKGFKEDDKYYGLQTKFTIQTENYSHKQLTNNAEEIIKRIPTAPK  
 AKWGSTTNGLTPEQQKEYYLSKVGETFTMKAFMEADDILSQVNRNSQKIIHVHVDGVPTRSYAINNFK  
 LGASYESQFEQMKNGYLKNSNELLTDKPEDIKNGESYFLFPLDSYQTQIIISGNLQKLHYLDLNLNY  
 PKGTIYRNGPVKEHGTPTKLYINSLKQKNYDIFNFGIDISGFRQVYNEEYKKNQDGTFOKLKEEAFKL  
 SDGEITELMRSFSSKPEYYTPIVTSADTSNNEILSKIQQQFETILTENSIVNGTIEDPMDKINLQL  
 40 GNGQTLQPSDYTLQNDGSMKDGIAATGGPNNDGGILKGVKLEYIGNKLYVRGLNLGEGQKVTLTYDV  
 KLDDSFISNKFYDTNGRDTLNPKSEDPNLTLDPIPKIRDVREYPTITIKNEKKLGEIEFIKVDKDNN  
 KLLKLGATFELQEFNEDYKLYLPIKNNNSKVVTGENGKISYKDLKDGKYQLIEAVSPEDYQKITNKPI  
 LTFEVVKGSIKNI IAVNKQISEYHEEGDKHLITNTHIPPKGI IPMTGGKGILSFILIGAMMSIAGGI  
YIWKRYKKSSDMSIKKD

45

GBS 67 contains a C-terminus transmembrane region which is indicated by the underlined region closest to the C-terminus of SEQ ID NO: 88 above. In one embodiment, one or more amino acids from the transmembrane region is removed and or the amino acid is truncated before the transmembrane region. An example of such a GBS 67 fragment is set forth below as SEQ ID NO: 89.

50



**SEQ ID NO: 89**

MRKYQKFSKILTLSLFCLSQIPLNTNVLGESTVPENGAKGKLVVKKTTDDQNKPLSKATFVLKTTAHPE  
 SKIEKVTAELTGEATFDNLI PGDYTLSEETAPEGYKKTNQ TWQVKVESNGKTTIQNSGDKNSTIGQNQ  
 EELDKQYPPTGIYEDTKESYKLEHVKGSV PNGKSEAKAVNPYSSEGEHIREI PEGTLSKR ISEVGDLA  
 5 HNKYKIELTVSGKTIVKPV DKQKPLDVVFVLDNSNSMNN DGP NFQRHNKAKKAAEALGTAVKDILGAN  
 SDNRVALVTYGS DIFDGRSVDVVKGFKEDDKYYGLQTKFTIQ TENYSHKQLTNNAEEI IKRIPT EAPK  
 AKWGSTTNGLTPEQQKEYYLSKVGETFTMKAFMEADDILSQVNRNSQKIIVHVTDGVPTRSYAINNFK  
 LGASYESQFEQMKNKYLNKSNFLLTDKPEDIKNGESYFLFPLDSYQTQIISGNLQKLHYLDLNLNY  
 10 PKGTIYRNGPVKEHGTPTKLYINSLKQKNYDIFNFGIDISGFRQVYNEEYKKNQDGT FQKLKEEAFKL  
 SDGEITELMRSFSSKPEYYTPIVTSADTSNNEILSKIQQQFETILT KENSIVNGTIEDPMDKINLQL  
 GNGQTLQPSDYTLQNDGSVMKDGIATGGPNNDGGILKGVKLEYIGNKLYVRGLNLGEGQKVTLTYDV  
 KLDDSFISNKFYDTNGRTTLNPKSEDPNTLRDFPIPKIRDVREYPTITIKNEKKLGEIEFIKVDKDNN  
 KLLLKGATFELQEFNEDYKLYLPIKNNNSKVVTGENGKISYKDLKDGKYQLIEAVSPEDYQKITNKPI  
 15 LTFEVVKGSIKNIIAVNKQISEYHEEGDKHLITNTHIPPKGIIPMTGGKGILS

GBS 67 contains an amino acid motif indicative of a cell wall anchor (an LPXTG motif):

**SEQ ID NO: 90** *IPMTG*. (shown in italics in SEQ ID NO: 88 above). In some recombinant host cell systems, it may be preferable to remove this motif to facilitate secretion of a recombinant GBS 67 protein from the host cell. Accordingly, in one preferred fragment of GBS 67 for use in the invention, the transmembrane and the cell wall anchor motif are removed from GBS 67. An example of such a GBS 67 fragment is set forth below as SEQ ID NO: 91.

**SEQ ID NO: 91**

MRKYQKFSKILTLSLFCLSQIPLNTNVLGESTVPENGAKGKLVVKKTTDDQNKPLSKATFVLKTTAHPE  
 SKIEKVTAELTGEATFDNLI PGDYTLSEETAPEGYKKTNQ TWQVKVESNGKTTIQNSGDKNSTIGQNQ  
 25 EELDKQYPPTGIYEDTKESYKLEHVKGSV PNGKSEAKAVNPYSSEGEHIREI PEGTLSKR ISEVGDLA  
 HNKYKIELTVSGKTIVKPV DKQKPLDVVFVLDNSNSMNN DGP NFQRHNKAKKAAEALGTAVKDILGAN  
 SDNRVALVTYGS DIFDGRSVDVVKGFKEDDKYYGLQTKFTIQ TENYSHKQLTNNAEEI IKRIPT EAPK  
 AKWGSTTNGLTPEQQKEYYLSKVGETFTMKAFMEADDILSQVNRNSQKIIVHVTDGVPTRSYAINNFK  
 LGASYESQFEQMKNKYLNKSNFLLTDKPEDIKNGESYFLFPLDSYQTQIISGNLQKLHYLDLNLNY  
 30 PKGTIYRNGPVKEHGTPTKLYINSLKQKNYDIFNFGIDISGFRQVYNEEYKKNQDGT FQKLKEEAFKL  
 SDGEITELMRSFSSKPEYYTPIVTSADTSNNEILSKIQQQFETILT KENSIVNGTIEDPMDKINLQL  
 GNGQTLQPSDYTLQNDGSVMKDGIATGGPNNDGGILKGVKLEYIGNKLYVRGLNLGEGQKVTLTYDV  
 KLDDSFISNKFYDTNGRTTLNPKSEDPNTLRDFPIPKIRDVREYPTITIKNEKKLGEIEFIKVDKDNN  
 KLLLKGATFELQEFNEDYKLYLPIKNNNSKVVTGENGKISYKDLKDGKYQLIEAVSPEDYQKITNKPI  
 35 LTFEVVKGSIKNIIAVNKQISEYHEEGDKHLITNTHIPPKGI

The compositions of the invention may also include combinations including one or more known GBS antigens in combination with GBS 80.

There is an upper limit to the number of GBS antigens which will be in the compositions of the invention. Preferably, the number of GBS antigens in a composition of the invention is less than 20, less than 19, less than 18, less than 17, less than 16, less than 15, less than 14, less than 13, less than 12, less than 11, less than 10, less than 9, less than 8, less than 7, less than 6, less than 5, less than 4, or less than 3. Still more preferably, the number of GBS antigens in a composition of the invention

is less than 6, less than 5, or less than 4. Still more preferably, the number of GBS antigens in a composition of the invention is 3.

The GBS antigens used in the invention are preferably isolated, i.e., separate and discrete, from the whole organism with which the molecule is found in nature or, when the polynucleotide or polypeptide is not found in nature, is sufficiently free of other biological macromolecules so that the polynucleotide or polypeptide can be used for its intended purpose.

#### Fusion Proteins

The GBS antigens used in the invention may be present in the composition as individual separate polypeptides, but it is preferred that at least two (*i.e.* 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, or 18) of the antigens are expressed as a single polypeptide chain (a “hybrid” or “fusion” polypeptide). Such fusion polypeptides offer two principal advantages: first, a polypeptide that may be unstable or poorly expressed on its own can be assisted by adding a suitable fusion partner that overcomes the problem; second, commercial manufacture is simplified as only one expression and purification need be employed in order to produce two polypeptides which are both antigenically useful.

The fusion polypeptide may comprise two or more polypeptide sequences from the group consisting of GBS 80, GBS 91, GBS 104, GBS 184, GBS 276, GBS 305, GBS 322, GBS 330, GBS 338, GBS 361, GBS 404, GBS 690 and GBS 691. Preferably, the polypeptide sequences are selected from the group consisting of GBS 80, GBS 104 and GBS 322. Most preferably, the fusion peptide includes a polypeptide sequence from GBS 80. Accordingly, the invention includes a fusion peptide comprising a first amino acid sequence and a second amino acid sequence, wherein said first and second amino acid sequences are selected from a GBS antigen or a fragment thereof of the above antigen group. Preferably, the first and second amino acid sequences in the fusion polypeptide comprise different epitopes.

#### **EXAMPLE 7: Examples of fragments for fusion proteins from GBS 80 with GBS 104, and GBS 322**

Examples of GBS fragments for fusion proteins are provided from GBS 322, GBS 104, and GBS 80. One example of a fragment of GBS 322 in a fusion protein is a 407 amino acid fragment with the signal peptide removed. Fragments of GBS 104 may also be incorporated in fusion proteins. An example of GBS 104 fragments includes an 830 amino acid fragment, a 359 amino acid fragment from near the N-terminus, a 581 amino acid fragment from near the N-terminus, and a 740 amino acid fragment from near the N-terminus. Examples of GBS 80 fragments include a 446 amino acid fragment and a 235 amino acid fragment. Table 13 below summarizes the examples of fragments for fusion proteins and their locations within the corresponding full length GBS protein.

Table 13: Active Maternal Immunization Assay using combinations of GBS 80 with GBS 104 and GBS 322

GBS	Size (AA)	SEQ ID NO	From ... to
322	407	92	25-432
104	830	96	28-858
104 N1	359	97	28-387
104 N2	581	98	28-609
104 N3	740	99	28-768
80	446	100	37-483
80N	235	101	37-272

5

Hybrids (or fusions) consisting of amino acid sequences from two, three, four, five, six, seven, eight, nine, or ten GBS antigens are preferred. In particular, hybrids consisting of amino acid sequences from two, three, four, or five GBS antigens are preferred.

10 Different hybrid polypeptides may be mixed together in a single formulation. Within such combinations, a GBS antigen may be present in more than one hybrid polypeptide and/or as a non-hybrid polypeptide. It is preferred, however, that an antigen is present either as a hybrid or as a non-hybrid, but not as both.

Hybrid polypeptides can be represented by the formula  $\text{NH}_2\text{-A-}\{-\text{X-L-}\}_n\text{-B-COOH}$ , wherein: X is an amino acid sequence of a GBS antigen or a fragment thereof from the antigen group set forth above; L is an optional linker amino acid sequence; A is an optional N-terminal amino acid sequence; B is an optional C-terminal amino acid sequence; and  $n$  is 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14 or 15.

15 If a -X- moiety has a leader peptide sequence in its wild-type form, this may be included or omitted in the hybrid protein. In some embodiments, the leader peptides will be deleted except for that of the -X- moiety located at the N-terminus of the hybrid protein *i.e.* the leader peptide of  $X_1$  will be retained, but the leader peptides of  $X_2 \dots X_n$  will be omitted. This is equivalent to deleting all leader peptides and using the leader peptide of  $X_1$  as moiety -A-.

For each  $n$  instances of  $\{-\text{X-L-}\}$ , linker amino acid sequence -L- may be present or absent. For instance, when  $n=2$  the hybrid may be  $\text{NH}_2\text{-X}_1\text{-L}_1\text{-X}_2\text{-L}_2\text{-COOH}$ ,  $\text{NH}_2\text{-X}_1\text{-X}_2\text{-COOH}$ ,  $\text{NH}_2\text{-X}_1\text{-L}_1\text{-X}_2\text{-COOH}$ ,  $\text{NH}_2\text{-X}_1\text{-X}_2\text{-L}_2\text{-COOH}$ , *etc.* Linker amino acid sequence(s) -L- will typically be short (*e.g.* 20 or fewer amino acids *i.e.* 19, 18, 17, 16, 15, 14, 13, 12, 11, 10, 9, 8, 7, 6, 5, 4, 3, 2, 1). Examples comprise short peptide sequences which facilitate cloning, poly-glycine linkers (*i.e.* comprising  $\text{Gly}_n$  where  $n = 2, 3, 4, 5, 6, 7, 8, 9, 10$  or more), and histidine tags (*i.e.*  $\text{His}_n$  where  $n = 3, 4, 5, 6, 7, 8, 9, 10$  or more). Other suitable linker amino acid sequences will be apparent to those skilled in the art. A useful linker is GSGGGG, with the Gly-Ser dipeptide being formed from a

*Bam*HI restriction site, thus aiding cloning and manipulation, and the (Gly)<sub>4</sub> tetrapeptide being a typical poly-glycine linker.

-A- is an optional N-terminal amino acid sequence. This will typically be short (*e.g.* 40 or fewer amino acids *i.e.* 39, 38, 37, 36, 35, 34, 33, 32, 31, 30, 29, 28, 27, 26, 25, 24, 23, 22, 21, 20, 19, 18, 17, 16, 15, 14, 13, 12, 11, 10, 9, 8, 7, 6, 5, 4, 3, 2, 1). Examples include leader sequences to direct protein trafficking, or short peptide sequences which facilitate cloning or purification (*e.g.* histidine tags *i.e.* His<sub>*n*</sub>, where *n* = 3, 4, 5, 6, 7, 8, 9, 10 or more). Other suitable N-terminal amino acid sequences will be apparent to those skilled in the art. If X<sub>1</sub> lacks its own N-terminus methionine, -A- is preferably an oligopeptide (*e.g.* with 1, 2, 3, 4, 5, 6, 7 or 8 amino acids) which provides a N-terminus methionine.

-B- is an optional C-terminal amino acid sequence. This will typically be short (*e.g.* 40 or fewer amino acids *i.e.* 39, 38, 37, 36, 35, 34, 33, 32, 31, 30, 29, 28, 27, 26, 25, 24, 23, 22, 21, 20, 19, 18, 17, 16, 15, 14, 13, 12, 11, 10, 9, 8, 7, 6, 5, 4, 3, 2, 1). Examples include sequences to direct protein trafficking, short peptide sequences which facilitate cloning or purification (*e.g.* comprising histidine tags *i.e.* His<sub>*n*</sub>, where *n* = 3, 4, 5, 6, 7, 8, 9, 10 or more), or sequences which enhance protein stability. Other suitable C-terminal amino acid sequences will be apparent to those skilled in the art. Most preferably, *n* is 2 or 3.

**EXAMPLE 8: Active Maternal Immunization Assay using fusion proteins of Fragments of GBS 80, GBS 67, and GBS 322**

In this example, fusion proteins of GBS antigens was used in the Active Maternal Immunization Assay with an isolate challenge of different GBS strains. In these experiments, the challenge dose for the different GBS strains was sufficient to kill approximately 70 – 90% of unimmunized pups and is equal to 10 x LD 50% (where LD 50% is the statistically derived Median Lethal Dose). The maternal mice were immunized according to the Active Maternal Immunization Assay schedule described above with the fusion proteins of a GBS 80 antigen with GBS 322 antigen in the GBS strains set forth in Table 14 below. Survival % was observed with the GBS fusion proteins. As shown in Table 14, in this particular challenge study, the survival rates for the fusion proteins in all of the GBS strains achieved up to 79%.

Table 14: Active Maternal Immunization Assay using fusion proteins of GBS 80 with GBS 322

	COH1 (III)		CJB111 (V)		515 (Ia)		DK21 (II)		2603 (V)	
GBS	Dead/ treated	Survival %	Dead/ treated	Survival %	Dead/ treated	Survival %	Dead/ treated	Survival %	Dead/ treated	Survival %
80N-322	16/40	60	8/39	79	12/28	57	7/19	63	8/37	78
80	4/24	83								
PBS	35/40	12	27/35	23	32/39	18	31/40	22	33/40	17
80-322	12/27	55							12/38	68
80	0/33	100	28/40	30						
322									1/16	94
PBS	19/20	5	38/39	2	25/29	14			19/26	27

#### Nucleic Acids

The invention also provides nucleic acid encoding the GBS antigens and/or the hybrid fusion polypeptides of the invention. Furthermore, the invention provides nucleic acid which can hybridise to these nucleic acids, preferably under "high stringency" conditions (*e.g.* 65°C in a 0.1xSSC, 0.5% SDS solution).

Polypeptides of the invention can be prepared by various means (*e.g.* recombinant expression, purification from cell culture, chemical synthesis, *etc.*) and in various forms (*e.g.* native, fusions, non-glycosylated, lipidated, *etc.*). They are preferably prepared in substantially pure form (*i.e.* substantially free from other GAS or host cell proteins).

Nucleic acid according to the invention can be prepared in many ways (*e.g.* by chemical synthesis, from genomic or cDNA libraries, from the organism itself, *etc.*) and can take various forms (*e.g.* single stranded, double stranded, vectors, probes, *etc.*). They are preferably prepared in substantially pure form (*i.e.* substantially free from other GBS or host cell nucleic acids).

The term "nucleic acid" includes DNA and RNA, and also their analogues, such as those containing modified backbones (*e.g.* phosphorothioates, *etc.*), and also peptide nucleic acids (PNA), *etc.* The invention includes nucleic acid comprising sequences complementary to those described above (*e.g.* for antisense or probing purposes).

The invention also provides a process for producing a polypeptide of the invention, comprising the step of culturing a host cell transformed with nucleic acid of the invention under conditions which induce polypeptide expression.

The invention provides a process for producing a polypeptide of the invention, comprising the step of synthesising at least part of the polypeptide by chemical means.

The invention provides a process for producing nucleic acid of the invention, comprising the step of amplifying nucleic acid using a primer-based amplification method (*e.g.* PCR).

5       The invention provides a process for producing nucleic acid of the invention, comprising the step of synthesising at least part of the nucleic acid by chemical means.

#### Purification and Recombinant Expression

10       The GBS antigens of the invention may be isolated from *Streptococcus agalactiae*, or they may be recombinantly produced, for instance, in a heterologous host. Preferably, the GBS antigens are prepared using a heterologous host. The heterologous host may be prokaryotic (*e.g.* a bacterium) or eukaryotic. It is preferably *E.coli*, but other suitable hosts include *Bacillus subtilis*, *Vibrio cholerae*, *Salmonella typhi*, *Salmonella typhimurium*, *Neisseria lactamica*, *Neisseria cinerea*, *Mycobacteria* (*e.g.* *M.tuberculosis*), yeasts, *etc.*

15       Recombinant production of polypeptides is facilitated by adding a tag protein to the GBS antigen to be expressed as a fusion protein comprising the tag protein and the GBS antigen. Such tag proteins can facilitate purification, detection and stability of the expressed protein. Tag proteins suitable for use in the invention include a polyarginine tag (Arg-tag), polyhistidine tag (His-tag), FLAG-tag, Strep-tag, c-myc-tag, S-tag, calmodulin-binding peptide, cellulose-binding domain, SBP-  
20 tag,, chitin-binding domain, glutathione S-transferase-tag (GST), maltose-binding protein, transcription termination anti-terminiation factor (NusA), *E. coli* thioredoxin (TrxA) and protein disulfide isomerase I (DsbA). Preferred tag proteins include His-tag and GST. A full discussion on the use of tag proteins can be found at Ref. 3.

25       After purification, the tag proteins may optionally be removed from the expressed fusion protein, *i.e.*, by specifically tailored enzymatic treatments known in the art. Commonly used proteases include enterokinase, tobacco etch virus (TEV), thrombin, and factor X<sub>a</sub>.

#### GBS polysaccharides

30       The compositions of the invention may be further improved by including GBS polysaccharides. Preferably, the GBS antigen and the saccharide each contribute to the immunological response in a recipient. The combination is particularly advantageous where the saccharide and polypeptide provide protection from different GBS serotypes.

35       The combined antigens may be present as a simple combination where separate saccharide and polypeptide antigens are administered together, or they may be present as a conjugated combination, where the saccharide and polypeptide antigens are covalently linked to each other.

Thus the invention provides an immunogenic composition comprising (i) one or more GBS polypeptide antigens and (ii) one or more GBS saccharide antigens. The polypeptide and the polysaccharide may advantageously be covalently linked to each other to form a conjugate.

Between them, the combined polypeptide and saccharide antigens preferably cover (or provide protection from) two or more GBS serotypes (*e.g.* 2, 3, 4, 5, 6, 7, 8 or more serotypes). The serotypes of the polypeptide and saccharide antigens may or may not overlap. For example, the polypeptide might protect against serogroup II or V, while the saccharide protects against either serogroups Ia, Ib, or III. Preferred combinations protect against the following groups of serotypes: (1) serotypes Ia and Ib, (2) serotypes Ia and II, (3) serotypes Ia and III, (4) serotypes Ia and IV, (5) serotypes Ia and V, (6) serotypes Ia and VI, (7) serotypes Ia and VII, (8) serotypes Ia and VIII, (9) serotypes Ib and II, (10) serotypes Ib and III, (11) serotypes Ib and IV, (12) serotypes Ib and V, (13) serotypes Ib and VI, (14) serotypes Ib and VII, (15) serotypes Ib and VIII, (16) serotypes II and III, (17) serotypes II and IV, (18) serotypes II and V, (19) serotypes II and VI, (20) serotypes II and VII, (21) serotypes II and VIII, (22) serotypes III and IV, (23) serotypes III and V, (24) serotypes III and VI, (25) serotypes III and VII, (26) serotypes III and VIII, (27) serotypes IV and V, (28) serotypes IV and VI, (29) serotypes IV and VII, (30) serotypes IV and VIII, (31) serotypes V and VI, (32) serotypes V and VII, (33) serotypes V and VIII, (34) serotypes VI and VII, (35) serotypes VI and VIII, and (36) serotypes VII and VIII.

Still more preferably, the combinations protect against the following groups of serotypes: (1) serotypes Ia and II, (2) serotypes Ia and V, (3) serotypes Ib and II, (4) serotypes Ib and V, (5) serotypes III and II, and (6) serotypes III and V. Most preferably, the combinations protect against serotypes III and V.

Protection against serotypes II and V is preferably provided by polypeptide antigens. Protection against serotypes Ia, Ib and/or III may be polypeptide or saccharide antigens.

In one embodiment, the immunogenic composition comprises a GBS saccharide antigen and at least two GBS polypeptide antigens or fragments thereof, wherein said GBS saccharide antigen comprises a saccharide selected from GBS serotype Ia, Ib, and III, and wherein said GBS polypeptide antigens comprise a combination of at least two polypeptide or a fragment thereof selected from the antigen group consisting of GBS 80, GBS 91, GBS 104, GBS 184, GBS 276, GBS 305, GBS 322, GBS 330, GBS 338, GBS 361, GBS 404, GBS 690, and GBS 691. Preferably, the combination includes one or more of GBS 80, GBS 104 and GBS 322. Still more preferably, the combination includes GBS 80 or a fragment thereof.

In certain embodiments, the compositions of the invention do not include a GBS polysaccharide. In certain embodiments, the combination does not include one or more of the GBS antigens selected from the group consisting of GBS 4, GBS 22, GBS 85, GBS 338 and GBS 361.

Immunogenic compositions and medicaments

Compositions of the invention are preferably immunogenic compositions, and are more preferably vaccine compositions. The pH of the composition is preferably between 6 and 8, preferably about 7. The pH may be maintained by the use of a buffer. The composition may be  
5 sterile and/or pyrogen-free. The composition may be isotonic with respect to humans.

Vaccines according to the invention may either be prophylactic (*i.e.* to prevent infection) or therapeutic (*i.e.* to treat infection), but will typically be prophylactic. Accordingly, the invention includes a method for the therapeutic or prophylactic treatment of a *Streptococcus agalactiae* infection in an animal susceptible to streptococcal infection comprising administering to said animal a  
10 therapeutic or prophylactic amount of the immunogenic compositions of the invention.

The invention also provides a composition of the invention for use as a medicament. The medicament is preferably able to raise an immune response in a mammal (*i.e.* it is an immunogenic composition) and is more preferably a vaccine.

The invention also provides the use of the compositions of the invention in the manufacture of  
15 a medicament for raising an immune response in a mammal. The medicament is preferably a vaccine.

The invention also provides for a kit comprising a first component comprising a combination of GBS antigens.

The invention also provides a delivery device pre-filled with the immunogenic compositions of the invention.

20 The invention also provides a method for raising an immune response in a mammal comprising the step of administering an effective amount of a composition of the invention. The immune response is preferably protective and preferably involves antibodies and/or cell-mediated immunity. The method may raise a booster response.

The mammal is preferably a human. Where the vaccine is for prophylactic use, the human is  
25 preferably a female (either of child bearing age or a teenager). Alternatively, the human may be elderly (e.g., over the age of 50, 55, 60, 65, 70 or 75) and may have an underlying disease such as diabetes or cancer. Where the vaccine is for therapeutic use, the human is preferably a pregnant female or an elderly adult.

30 These uses and methods are preferably for the prevention and/or treatment of a disease caused by *Streptococcus agalactiae*. The compositions may also be effective against other streptococcal bacteria.

One way of checking efficacy of therapeutic treatment involves monitoring GBS infection after administration of the composition of the invention. One way of checking efficacy of prophylactic treatment involves monitoring immune responses against the GBS antigens in the  
35 compositions of the invention after administration of the composition.



Compositions of the invention will generally be administered directly to a patient. Direct delivery may be accomplished by parenteral injection (*e.g.* subcutaneously, intraperitoneally, intradermally, intravenously, intramuscularly, or to the interstitial space of a tissue), or by rectal, oral (*e.g.* tablet, spray), vaginal, topical, transdermal {*e.g.* see ref. 4} or transcutaneous {*e.g.* see refs. 5 & 6}, intranasal {*e.g.* see ref. 7}, ocular, aural, pulmonary or other mucosal administration.

The invention may be used to elicit systemic and/or mucosal immunity.

Dosage treatment can be a single dose schedule or a multiple dose schedule. Multiple doses may be used in a primary immunisation schedule and/or in a booster immunisation schedule. In a multiple dose schedule the various doses may be given by the same or different routes *e.g.* a parenteral prime and mucosal boost, a mucosal prime and parenteral boost, *etc.*

The compositions of the invention may be prepared in various forms. For example, the compositions may be prepared as injectables, either as liquid solutions or suspensions. Solid forms suitable for solution in, or suspension in, liquid vehicles prior to injection can also be prepared (*e.g.* a lyophilised composition). The composition may be prepared for topical administration *e.g.* as an ointment, cream or powder. The composition may be prepared for oral administration *e.g.* as a tablet or capsule, as a spray, or as a syrup (optionally flavoured). The composition may be prepared for pulmonary administration *e.g.* as an inhaler, using a fine powder or a spray. The composition may be prepared as a suppository or pessary. The composition may be prepared for nasal, aural or ocular administration *e.g.* as drops. The composition may be in kit form, designed such that a combined composition is reconstituted just prior to administration to a patient. Such kits may comprise one or more antigens in liquid form and one or more lyophilised antigens.

Immunogenic compositions used as vaccines comprise an immunologically effective amount of antigen(s), as well as any other components, as needed. By 'immunologically effective amount', it is meant that the administration of that amount to an individual, either in a single dose or as part of a series, is effective for treatment or prevention. This amount varies depending upon the health and physical condition of the individual to be treated, age, the taxonomic group of individual to be treated (*e.g.* non-human primate, primate, *etc.*), the capacity of the individual's immune system to synthesise antibodies, the degree of protection desired, the formulation of the vaccine, the treating doctor's assessment of the medical situation, and other relevant factors. It is expected that the amount will fall in a relatively broad range that can be determined through routine trials.

#### Further Components of the Composition

The composition of the invention will typically, in addition to the components mentioned above, comprise one or more 'pharmaceutically acceptable carriers', which include any carrier that does not itself induce the production of antibodies harmful to the individual receiving the

composition. Suitable carriers are typically large, slowly metabolised macromolecules such as proteins, polysaccharides, polylactic acids, polyglycolic acids, polymeric amino acids, amino acid copolymers, and lipid aggregates (such as oil droplets or liposomes). Such carriers are well known to those of ordinary skill in the art. The vaccines may also contain diluents, such as water, saline, glycerol, *etc.* Additionally, auxiliary substances, such as wetting or emulsifying agents, pH buffering substances, and the like, may be present. A thorough discussion of pharmaceutically acceptable excipients is available in reference 8.

Vaccines of the invention may be administered in conjunction with other immunoregulatory agents. In particular, compositions will usually include an adjuvant.

Preferred further adjuvants include, but are not limited to, one or more of the following set forth below:

A. Mineral Containing Compositions

Mineral containing compositions suitable for use as adjuvants in the invention include mineral salts, such as aluminium salts and calcium salts. The invention includes mineral salts such as hydroxides (*e.g.* oxyhydroxides), phosphates (*e.g.* hydroxyphosphates, orthophosphates), sulphates, *etc.* (*e.g.* see chapters 8 & 9 of ref. 9}), or mixtures of different mineral compounds, with the compounds taking any suitable form (*e.g.* gel, crystalline, amorphous, *etc.*), and with adsorption being preferred. The mineral containing compositions may also be formulated as a particle of metal salt. See ref. 10.

B. Oil-Emulsions

Oil-emulsion compositions suitable for use as adjuvants in the invention include squalene-water emulsions, such as MF59 (5% Squalene, 0.5% Tween 80, and 0.5% Span 85, formulated into submicron particles using a microfluidizer). See WO90/14837. See also, Frey et al., "Comparison of the safety, tolerability, and immunogenicity of a MF59-adjuvanted influenza vaccine and a non-adjuvanted influenza vaccine in non-elderly adults", *Vaccine* (2003) 21:4234 – 4237.

Particularly preferred adjuvants for use in the compositions are submicron oil-in-water emulsions. Preferred submicron oil-in-water emulsions for use herein are squalene/water emulsions optionally containing varying amounts of MTP-PE, such as a submicron oil-in-water emulsion containing 4-5% w/v squalene, 0.25-1.0% w/v Tween 80™ (polyoxyethylthylenesorbitan monooleate), and/or 0.25-1.0% Span 85™ (sorbitan trioleate), and, optionally, N-acetylmuramyl-L-alanyl-D-isoglutaminyl-L-alanine-2-(1'-2'-dipalmitoyl-*sn*-glycero-3-hydroxyphosphoryloxy)-ethylamine (MTP-PE), for example, the submicron oil-in-water emulsion known as "MF59" (International Publication No. WO 90/14837; U.S. Patent Nos. 6,299,884 and 6,451,325, incorporated herein by reference in their entireties; and Ott et al.,

"MF59 -- Design and Evaluation of a Safe and Potent Adjuvant for Human Vaccines" in *Vaccine Design: The Subunit and Adjuvant Approach* (Powell, M.F. and Newman, M.J. eds.) Plenum Press, New York, 1995, pp. 277-296). MF59 contains 4-5% w/v Squalene (e.g., 4.3%), 0.25-0.5% w/v Tween 80™, and 0.5% w/v Span 85™ and optionally contains various amounts of MTP-PE, formulated into submicron particles using a microfluidizer such as Model 110Y microfluidizer (Microfluidics, Newton, MA). For example, MTP-PE may be present in an amount of about 0-500 µg/dose, more preferably 0-250 µg/dose and most preferably, 0-100 µg/dose. As used herein, the term "MF59-0" refers to the above submicron oil-in-water emulsion lacking MTP-PE, while the term MF59-MTP denotes a formulation that contains MTP-PE. For instance, "MF59-100" contains 100 µg MTP-PE per dose, and so on. MF69, another submicron oil-in-water emulsion for use herein, contains 4.3% w/v squalene, 0.25% w/v Tween 80™, and 0.75% w/v Span 85™ and optionally MTP-PE. Yet another submicron oil-in-water emulsion is MF75, also known as SAF, containing 10% squalene, 0.4% Tween 80™, 5% pluronic-blocked polymer L121, and thr-MDP, also microfluidized into a submicron emulsion. MF75-MTP denotes an MF75 formulation that includes MTP, such as from 100-400 µg MTP-PE per dose.

Submicron oil-in-water emulsions, methods of making the same and immunostimulating agents, such as muramyl peptides, for use in the compositions, are described in detail in International Publication No. WO 90114837 and U.S. Patent Nos. 6,299,884 and 6,451,325, incorporated herein by reference in their entireties.

Complete Freund's adjuvant (CFA) and incomplete Freund's adjuvant (IFA) may also be used as adjuvants in the invention.

#### C. Saponin Formulations

Saponin formulations, may also be used as adjuvants in the invention. Saponins are a heterologous group of sterol glycosides and triterpenoid glycosides that are found in the bark, leaves, stems, roots and even flowers of a wide range of plant species. Saponin from the bark of the *Quillaia saponaria* Molina tree have been widely studied as adjuvants. Saponin can also be commercially obtained from *Smilax ornata* (sarsapilla), *Gypsophilla paniculata* (brides veil), and *Saponaria officianalis* (soap root). Saponin adjuvant formulations include purified formulations, such as QS21, as well as lipid formulations, such as ISCOMs.

Saponin compositions have been purified using High Performance Thin Layer Chromatography (HP-LC) and Reversed Phase High Performance Liquid Chromatography (RP-HPLC). Specific purified fractions using these techniques have been identified, including QS7, QS17, QS18, QS21, QH-A, QH-B and QH-C. Preferably, the saponin is QS21. A method of production of

QS21 is disclosed in U.S. Patent No. 5,057,540. Saponin formulations may also comprise a sterol, such as cholesterol (see WO 96/33739).

Combinations of saponins and cholesterol can be used to form unique particles called Immunostimulating Complexs (ISCOMs). ISCOMs typically also include a phospholipid such as phosphatidylethanolamine or phosphatidylcholine. Any known saponin can be used in ISCOMs. Preferably, the ISCOM includes one or more of Quil A, QHA and QHC. ISCOMs are further described in EP 0 109 942, WO 96/11711 and WO 96/33739. Optionally, the ISCOMS may be devoid of additional detergent. See ref. 11.

A review of the development of saponin based adjuvants can be found at ref. 12.

10 C. Virosomes and Virus Like Particles (VLPs)

Virosomes and Virus Like Particles (VLPs) can also be used as adjuvants in the invention. These structures generally contain one or more proteins from a virus optionally combined or formulated with a phospholipid. They are generally non-pathogenic, non-replicating and generally do not contain any of the native viral genome. The viral proteins may be recombinantly produced or isolated from whole viruses. These viral proteins suitable for use in virosomes or VLPs include proteins derived from influenza virus (such as HA or NA), Hepatitis B virus (such as core or capsid proteins), Hepatitis E virus, measles virus, Sindbis virus, Rotavirus, Foot-and-Mouth Disease virus, Retrovirus, Norwalk virus, human Papilloma virus, HIV, RNA-phages, Q $\beta$ -phage (such as coat proteins), GA-phage, fr-phage, AP205 phage, and Ty (such as retrotransposon Ty protein p1). VLPs are discussed further in WO 03/024480, WO 03/024481, and Refs. 13, 14, 15 and 16. Virosomes are discussed further in, for example, Ref. 17

D. Bacterial or Microbial Derivatives

Adjuvants suitable for use in the invention include bacterial or microbial derivatives such as:

(1) *Non-toxic derivatives of enterobacterial lipopolysaccharide (LPS)*

Such derivatives include Monophosphoryl lipid A (MPL) and 3-O-deacylated MPL (3dMPL). 3dMPL is a mixture of 3 De-O-acylated monophosphoryl lipid A with 4, 5 or 6 acylated chains. A preferred "small particle" form of 3 De-O-acylated monophosphoryl lipid A is disclosed in EP 0 689 454. Such "small particles" of 3dMPL are small enough to be sterile filtered through a 0.22 micron membrane (see EP 0 689 454). Other non-toxic LPS derivatives include monophosphoryl lipid A mimics, such as aminoalkyl glucosaminide phosphate derivatives *e.g.* RC-529. See Ref. 18.

(2) *Lipid A Derivatives*

Lipid A derivatives include derivatives of lipid A from *Escherichia coli* such as OM-174. OM-174 is described for example in Ref. 19 and 20.

(3) *Immunostimulatory oligonucleotides*

Immunostimulatory oligonucleotides suitable for use as adjuvants in the invention include nucleotide sequences containing a CpG motif (a sequence containing an unmethylated cytosine  
5 followed by guanosine and linked by a phosphate bond). Bacterial double stranded RNA or oligonucleotides containing palindromic or poly(dG) sequences have also been shown to be immunostimulatory.

The CpG's can include nucleotide modifications/analogues such as phosphorothioate modifications and can be double-stranded or single-stranded. Optionally, the guanosine may be  
10 replaced with an analog such as 2'-deoxy-7-deazaguanosine. See ref. 21, WO 02/26757 and WO 99/62923 for examples of possible analog substitutions. The adjuvant effect of CpG oligonucleotides is further discussed in Refs. 22, 23, WO 98/40100, U.S. Patent No. 6,207,646, U.S. Patent No. 6,239,116, and U.S. Patent No. 6,429,199.

The CpG sequence may be directed to TLR9, such as the motif GTCGTT or TTCGTT. See  
15 ref. 24. The CpG sequence may be specific for inducing a Th1 immune response, such as a CpG-A ODN, or it may be more specific for inducing a B cell response, such as a CpG-B ODN. CpG-A and CpG-B ODNs are discussed in refs. 25, 26 and WO 01/95935. Preferably, the CpG is a CpG-A ODN.

Preferably, the CpG oligonucleotide is constructed so that the 5' end is accessible for receptor  
20 recognition. Optionally, two CpG oligonucleotide sequences may be attached at their 3' ends to form "immunomers". See, for example, refs. 27, 28, 29 and WO 03/035836.

(4) *ADP-ribosylating toxins and detoxified derivatives thereof.*

Bacterial ADP-ribosylating toxins and detoxified derivatives thereof may be used as  
25 adjuvants in the invention. Preferably, the protein is derived from *E. coli* (i.e., *E. coli* heat labile enterotoxin "LT"), cholera ("CT"), or pertussis ("PT"). The use of detoxified ADP-ribosylating toxins as mucosal adjuvants is described in WO 95/17211 and as parenteral adjuvants in WO 98/42375. Preferably, the adjuvant is a detoxified LT mutant such as LT-K63.

E. Human Immunomodulators

Human immunomodulators suitable for use as adjuvants in the invention include cytokines,  
30 such as interleukins (e.g. IL-1, IL-2, IL-4, IL-5, IL-6, IL-7, IL-12, etc.), interferons (e.g. interferon- $\gamma$ ), macrophage colony stimulating factor, and tumor necrosis factor.

F. Bioadhesives and Mucoadhesives

Bioadhesives and mucoadhesives may also be used as adjuvants in the invention. Suitable  
bioadhesives include esterified hyaluronic acid microspheres (Ref. 30) or mucoadhesives such as cross-linked derivatives of poly(acrylic acid), polyvinyl alcohol, polyvinyl pyrrolidone,

polysaccharides and carboxymethylcellulose. Chitosan and derivatives thereof may also be used as adjuvants in the invention. E.g., ref. 31.

G. Microparticles

Microparticles may also be used as adjuvants in the invention. Microparticles (*i.e.* a particle  
5 of ~100nm to ~150µm in diameter, more preferably ~200nm to ~30µm in diameter, and most preferably ~500nm to ~10µm in diameter) formed from materials that are biodegradable and non-toxic (*e.g.* a poly(α-hydroxy acid), a polyhydroxybutyric acid, a polyorthoester, a polyanhydride, a polycaprolactone, *etc.*), with poly(lactide-co-glycolide) are preferred, optionally treated to have a negatively-charged surface (*e.g.* with SDS) or a positively-charged surface (*e.g.* with a cationic  
10 detergent, such as CTAB).

H. Liposomes

Examples of liposome formulations suitable for use as adjuvants are described in U.S. Patent No. 6,090,406, U.S. Patent No. 5,916,588, and EP 0 626 169.

I. Polyoxyethylene ether and Polyoxyethylene Ester Formulations

15 Adjuvants suitable for use in the invention include polyoxyethylene ethers and polyoxyethylene esters. Ref. 32. Such formulations further include polyoxyethylene sorbitan ester surfactants in combination with an octoxynol (Ref. 33) as well as polyoxyethylene alkyl ethers or ester surfactants in combination with at least one additional non-ionic surfactant such as an octoxynol (Ref. 34).

20 Preferred polyoxyethylene ethers are selected from the following group: polyoxyethylene-9-lauryl ether (laureth 9), polyoxyethylene-9-stearyl ether, polyoxyethylene-8-stearyl ether, polyoxyethylene-4-lauryl ether, polyoxyethylene-35-lauryl ether, and polyoxyethylene-23-lauryl ether.

J. Polyphosphazene (PCPP)

25 PCPP formulations are described, for example, in Ref. 35 and 36.

K. Muramyl peptides

Examples of muramyl peptides suitable for use as adjuvants in the invention include N-acetyl-muramyl-L-threonyl-D-isoglutamine (thr-MDP), N-acetyl-normuramyl-L-alanyl-D-isoglutamine (nor-MDP), and N-acetylmuramyl-L-alanyl-D-isoglutaminyl-L-alanine-2-(1'-2'-dipalmitoyl-*sn*-glycero-3-  
30 hydroxyphosphoryloxy)-ethylamine MTP-PE).

L. Imidazoquinolone Compounds.

Examples of imidazoquinolone compounds suitable for use as adjuvants in the invention include Imiquamod and its homologues, described further in Ref. 37 and 38.

The invention may also comprise combinations of aspects of one or more of the adjuvants identified above. For example, the following adjuvant compositions may be used in the invention:

- (1) a saponin and an oil-in-water emulsion (ref. 39);
- 5 (2) a saponin (e.g., QS21) + a non-toxic LPS derivative (e.g., 3dMPL) (see WO 94/00153);
- (3) a saponin (e.g., QS21) + a non-toxic LPS derivative (e.g., 3dMPL) + a cholesterol;
- (4) a saponin (e.g. QS21) + 3dMPL + IL-12 (optionally + a sterol) (Ref. 40);
- (5) combinations of 3dMPL with, for example, QS21 and/or oil-in-water emulsions (Ref. 10 41);
- (6) SAF, containing 10% Squalane, 0.4% Tween 80, 5% pluronic-block polymer L121, and thr-MDP, either microfluidized into a submicron emulsion or vortexed to generate a larger particle size emulsion.

(7) Ribi<sup>TM</sup> adjuvant system (RAS), (Ribi Immunochem) containing 2% Squalene, 0.2% Tween 80, and one or more bacterial cell wall components from the group consisting of 15 monophosphorylipid A (MPL), trehalose dimycolate (TDM), and cell wall skeleton (CWS), preferably MPL + CWS (Detox<sup>TM</sup>); and

(8) one or more mineral salts (such as an aluminum salt) + a non-toxic derivative of LPS (such as 3dPML).

20 Aluminium salts and MF59 are preferred adjuvants for parenteral immunisation. Mutant bacterial toxins are preferred mucosal adjuvants.

The composition may include an antibiotic.

### ***Further antigens***

The compositions of the invention may further comprise one or more additional non-GBS 25 antigens, including additional bacterial, viral or parasitic antigens.

In another embodiment, the GBS antigen combinations of the invention are combined with one or more additional, non-GBS antigens suitable for use in a vaccine designed to protect elderly or immunocomprised individuals. For example, the GBS antigen combinations may be combined with an antigen derived from the group consisting of *Enterococcus faecalis*, *Staphylococcus aureus*, 30 *Staphylococcus epidermis*, *Pseudomonas aeruginosa*, *Legionella pneumophila*, *Listeria monocytogenes*, *Neisseria meningitides*, influenza, and Parainfluenza virus ('PIV').

Where a saccharide or carbohydrate antigen is used, it is preferably conjugated to a carrier protein in order to enhance immunogenicity {e.g. refs. 42 to 51}. Preferred carrier proteins are bacterial toxins or toxoids, such as diphtheria or tetanus toxoids. The CRM<sub>197</sub> diphtheria toxoid is 35 particularly preferred {52}. Other carrier polypeptides include the *N.meningitidis* outer membrane

protein {53}, synthetic peptides {54, 55}, heat shock proteins {56, 57}, pertussis proteins {58, 59}, protein D from *H.influenzae* {60}, cytokines {61}, lymphokines, hormones, growth factors, toxin A or B from *C.difficile* {62}, iron-uptake proteins {63}, *etc.* Where a mixture comprises capsular saccharides from both serogroups A and C, it may be preferred that the ratio (w/w) of MenA  
 5 saccharide:MenC saccharide is greater than 1 (*e.g.* 2:1, 3:1, 4:1, 5:1, 10:1 or higher). Different saccharides can be conjugated to the same or different type of carrier protein. Any suitable conjugation reaction can be used, with any suitable linker where necessary.

Toxic protein antigens may be detoxified where necessary *e.g.* detoxification of pertussis toxin by chemical and/or genetic means.

10 Where a diphtheria antigen is included in the composition it is preferred also to include tetanus antigen and pertussis antigens. Similarly, where a tetanus antigen is included it is preferred also to include diphtheria and pertussis antigens. Similarly, where a pertussis antigen is included it is preferred also to include diphtheria and tetanus antigens.

15 Antigens in the composition will typically be present at a concentration of at least 1 µg/ml each. In general, the concentration of any given antigen will be sufficient to elicit an immune response against that antigen.

20 As an alternative to using protein antigens in the composition of the invention, nucleic acid encoding the antigen may be used (*e.g.* refs. 64 to 72). Protein components of the compositions of the invention may thus be replaced by nucleic acid (preferably DNA *e.g.* in the form of a plasmid) that encodes the protein.

### Definitions

The term “comprising” means “including” as well as “consisting” *e.g.* a composition “comprising” X may consist exclusively of X or may include something additional *e.g.* X + Y.

25 The term “about” in relation to a numerical value *x* means, for example,  $x \pm 10\%$ .

References to a percentage sequence identity between two amino acid sequences means that, when aligned, that percentage of amino acids are the same in comparing the two sequences. This alignment and the percent homology or sequence identity can be determined using software programs known in the art, for example those described in section 7.7.18 of reference 73. A preferred  
 30 alignment is determined by the Smith-Waterman homology search algorithm using an affine gap search with a gap open penalty of 12 and a gap extension penalty of 2, BLOSUM matrix of 62. The Smith-Waterman homology search algorithm is disclosed in reference 74.



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## WHAT IS CLAIMED IS:

1. A composition comprising a combination of two or more GBS antigens, wherein said combination includes GBS 80 or a fragment thereof or a polypeptide sequence having 50% or greater sequence identity thereto.
2. The composition of claim 1, wherein said combination of GBS antigens demonstrates improved immunogenicity as measured by the Active Maternal Immunization Assay, wherein said Active Maternal Immunization Assay measures serum titers of female mice during an immunization schedule and percent survival rate of pups after challenge.
3. The composition of claim 2, wherein the percent survival rate of challenged pups is at least 2 percentage points higher than the percent survival rate of challenged pups from female mice immunized with a single non-GBS 80 antigen.
4. The composition of claim 1, wherein said combination consists of two GBS antigens.
5. The composition of claim 1, wherein said combination consists of three GBS antigens.
6. The composition of claim 1, wherein said combination consists of four GBS antigens.
7. The composition of claim 1, wherein said combination consists of five GBS antigens.
8. The composition of claim 1, wherein GBS 80 comprises the amino acid sequence of SEQ ID NO 2 or an immunogenic fragment thereof.
9. The composition of claim 1, wherein the fragment of GBS 80 comprises the amino acid sequence selected from the group consisting of SEQ ID NOS: 3, 4, 5, 6, 7, 8, and 9.
10. The composition of claim 1, said combination consisting of two to thirteen GBS antigens selected from the group consisting of GBS 80, GBS 91, GBS 104, GBS 184, GBS 276, GBS 305, GBS 322, GBS 330, GBS 338, GBS 361, GBS 404, GBS 690, and GBS 691.
11. The composition of claim 1, said combination including GBS 80, GBS 104 and GBS 322.
12. The composition of claim 1, said combination including GBS 80, GBS 104, GBS 276 and GBS 322.
13. The combination of claim 1 wherein said combination comprises at least one of GBS 91, GBS 104, GBS 184, GBS 276, GBS 305, GBS 322, GBS 330, GBS 338, GBS 361, GBS 404, GBS 690, or GBS 691.

14. A fusion protein comprising a portion of a GBS 80 antigen and a portion of at least one GBS antigen.
15. The fusion protein of claim 14 wherein said at least one GBS antigen is selected from the group consisting of GBS 91, GBS 104, GBS 184, GBS 276, GBS 305, GBS 322, GBS 330, GBS 338, GBS 361, GBS 404, GBS 690, or GBS 691.
16. The fusion protein of claim 15 wherein said at least one GBS antigen is GBS 322.
17. The fusion protein of claim 16 consisting essentially of a GBS 80 antigen and a GBS 322 antigen.
18. A method for the therapeutic or prophylactic treatment of GBS infection in an animal susceptible to GBS infection comprising administering to said animal a therapeutic or prophylactic amount of the composition of claim 1.
19. A method for the manufacture of a medicament for raising an immune response against GBS comprising combining a GBS 80 antigen or fragment thereof with at least one GBS polypeptide antigen.
20. The method of claim 19 wherein said at least one GBS polypeptide antigen comprises a polypeptide or fragment thereof selected from the antigen group consisting of GBS 91, GBS 104, GBS 184, GBS 276, GBS 305, GBS 322, GBS 330, GBS 338, GBS 361, GBS 404, GBS 690, and GBS 691.
21. Use of the compositions of any one of claims 1-17 in the preparation of a medicament for treatment of GBS infection.

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(54) Title: IMMUNOGENIC COMPOSITIONS FOR *STREPTOCOCCUS AGALACTIAE*

(57) Abstract: This application relates to Group B Streptococcus ("GBS") vaccines comprising combinations of GBS polypeptide antigens where the polypeptides contribute to the immunological response in a recipient. Preferably, the compositions of the invention comprise a combination of two or more GBS antigens, wherein said combination includes GBS 80 or a fragment thereof. In one embodiment, the combination may consist of two to thirteen GBS antigens selected from an antigen group consisting of GBS 80, GBS 91, GBS 104, GBS 184, GBS 276, GBS 305, GBS 322, GBS 330, GBS 338, GBS 361, GBS 404, GBS 690, and GBS 691.



WO 2005/028618 A3

# INTERNATIONAL SEARCH REPORT

International application No.

PCT/US04/30032

<b>A. CLASSIFICATION OF SUBJECT MATTER</b> IPC(7) : A61K 39/385, 39/116, 39/00, 39/02, 39/38, 39/09 US CL : 424/197.11, 203.1, 192.1, 190.1, 184.1, 244.1 According to International Patent Classification (IPC) or to both national classification and IPC		
<b>B. FIELDS SEARCHED</b> Minimum documentation searched (classification system followed by classification symbols) U.S. : 424/197.11, 203.1, 192.1, 190.1, 184.1, 244.1 Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched Electronic data base consulted during the international search (name of data base and, where practicable, search terms used) Please See Continuation Sheet		
<b>C. DOCUMENTS CONSIDERED TO BE RELEVANT</b>		
Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2004/041157 A2 (CHIRON CORPORATION) 21 May 2004 (21.05.2004), claims, and pages 4 and 5.	1-17
<input type="checkbox"/> Further documents are listed in the continuation of Box C. <input type="checkbox"/> See patent family annex.		
* Special categories of cited documents:		
"A"	document defining the general state of the art which is not considered to be of particular relevance	"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"E"	earlier application or patent published on or after the international filing date	"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"O"	document referring to an oral disclosure, use, exhibition or other means	"&" document member of the same patent family
"P"	document published prior to the international filing date but later than the priority date claimed	
Date of the actual completion of the international search 09 November 2005 (09.11.2005)		Date of mailing of the international search report <b>06 DEC 2005</b>
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US Commissioner for Patents P.O. Box 1450 Alexandria, Virginia 22313-1450 Facsimile No. (571) 273-3201		Authorized officer S. Devi, Ph.D. <i>Janice Ford</i> Telephone No. (571) 272-1600 <i>for</i>

# INTERNATIONAL SEARCH REPORT

International application No.

PCT/US04/30032

## Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:  
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☐ Claims Nos.:  
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☐ Claims Nos.:  
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

## Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:  
Please See Continuation Sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of any additional fees.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.: 1-17

- Remark on Protest
- |                          |   |
|--------------------------|---|
| <input type="checkbox"/> | The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.   |
| <input type="checkbox"/> | The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation. |
| <input type="checkbox"/> | No protest accompanied the payment of additional search fees.   |



## INTERNATIONAL SEARCH REPORT

International application No.  
PCT/US04/30032

### BOX III. OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING

This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees must be paid.

Group I, claim(s) 1-17, drawn to a composition comprising a combination of two or more GBS antigens comprising GBS 80 or a fragment thereof.

Group II, claim(s) 18, drawn to a method for the therapeutic or prophylactic treatment of GBS infection by administering the composition of invention I.

Group III, claim(s) 19-21, drawn to a method for the manufacture of a medicament by combining a GBS 80 antigen fragment thereof with at least one GBS polypeptide antigen.

The inventions listed as Groups I-III do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:

Inventions I-III lack unity. The special technical feature of invention I is a composition comprising a combination of two or more GBS antigens comprising GBS 80 or a fragment thereof. However, such a composition was already disclosed in the prior art. For instance, CHIRON CORPORATION (WO 2004/041157 A2) disclosed a composition comprising a combination of GBS 80 having the amino acid sequence of SEQ ID NO: 2 and GBS 322 antigen. Thus, the product of invention I does not define over the prior art. Although the product of invention I and the method of using the product of invention II and a method of making the product of invention III is a permitted combination under PCT Rule 13.2, in the instant case, since the product of invention I is already disclosed in the art, the special technical feature is not a unifying feature. Technically, the absence of special technical feature permits the separation of the method of using the product or the method of making the product from the product itself.

Continuation of B. FIELDS SEARCHED Item 3:

## INTERNATIONAL SEARCH REPORT

International application No.

PCT/US04/30032

DIALOG, WEST, MEDLINE, BIOSIS, EMBASE, Sequence databases  
GBS 80, SEQ ID NO: 2, inventors' names